Fabrication of Au-DNA-Au nanostructure with new-type DNA-Au conjugate

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ABSTRACT

Nano devices fabricated from DNA and nanoparticles found many important applications in both biotechnology and nanotechnology. Conventionally, DNA was linked to gold surfaces through a linker. In this study, DNAs were directly attached to gold surfaces through S-Au bonds by modifying 5’-end of DNA with a mercapto group. The pH of the reaction mixture played an important role in DNA-gold conjugate fabrication. The DNA-Au conjugates were successfully obtained in pure forms and, with the use of this new type conjugate, Au-DNA-Au nanostructure was prepared. The Au-DNA-Au nanostructure was characterized by AFM.

INTRODUCTION

DNA-modified gold colloidal particles attracted tremendous interests in recent years. Various genetic diagnostics technologies were developed and numerous nanostructures were fabricated. To immobilize DNA to gold surfaces, various mercapto-modified DNAs 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 paper, DNA-Au conjugates are fabricated as shown in Scheme 1. By using 5’-mercaptop-5’-deoxythymidine, DNA is directly attached to gold surfaces to obtain a new type DNA-Au conjugate. This new type DNA-Au conjugate showed good hybridisation specificity in Au-DNA-Au nanostructure fabrication.

MATERIALS AND METHODS

Preparation of Au-BSPP conjugate

Solutions of Au particles (average size 10 nm) were purchased from Sigma. Their conjugates with bis(p-sulfonatophenyl) phenyl phosphane (BSPP) were prepared according to literature.

Oligonucleotides.

The oligonucleotides used in this study were synthesized and purified as described previously. The sequences of the oligonucleotides were as follows. DNA1: 5’-HS-TACC AGGATTACGCCCTACA, DNA2: 5’-HS-TGTTAGCGG TAATCCTGGA, DNA3: 5’-HS-C6-TACCAGGATTAC CGCTCCA (bearing a C6 linker between its mercapto group and DNA backbone), DNA4: 5’-TACCAGGATTA CGCGTCCA (a native DNA for control).

Fabrication of DNA-Au conjugate

DNA-Au conjugates were prepared by mixing DNA (0.5 μM) and Au-BSPP conjugate (0.05 μM) in a 50 mM TBE buffer (pH 8.0 or pH 4.0) containing 0.1 M NaCl. The conjugate solution was shaken at ambient temperature for 24 h.

Electrophoretic Mobility-Shift Assay (EMSA).

DNA-Au conjugates were assayed by a 5.5% poly acrylamide gel. The gel was stained by Gelstar (Cambrex Co.) and imaged by Fuji Film FLA-3000G imaging analyzer.

Scheme 1. Fabrication of DNA–Au conjugate and Au-DNA-Au nanostructure.
Fabrication of Au-DNA-Au nanostructure

Gold substrate on mica (Au (111)) was incubated for 1 h in 0.1 μM DNA-SH (DNA1 or DNA2) solution in the presence of 10 μM octanethiol in PBS (-) buffer. The gold substrate was rinsed with water. The substrate was incubated in 0.01 μM DNA1-Au conjugate solution in 0.1 M NaCl for 3 h and rinsed by excess of water.

AFM Assay

The substrate was dried and subjected to AFM assay on an SPA300HV unit in tapping mode using DFM Probe SI-DF20 (from Seiko Instruments Co.).

RESULTS AND DISCUSSION

Electrophoresis was employed to monitor the formation of DNA-Au conjugates (Fig. 1). Both the change of mobility of Au particles and the disappearance of DNA bands provide good evidence for the DNA-Au conjugates formation. At pH 8, DNA1 or DNA4 doesn’t attach to gold particles (lanes 3 and 4), although conventional DNA-SH (DNA3) attaches to the gold particles (lane 2). When the pH was 4.0 or lower, however, both the DNA1 and DNA3 are effectively bound to the gold particles (lanes 5 and 6). Since DNA4 (without mercapto group) doesn’t attach to gold particles at any pH (lane 4, 7), the possibility of nonspecific attachment can be ruled out. The different behavior of the two types of DNA-SH (with or without the alkyl linker) is attributed to the electrostatic repulsion between the negatively charged Au-BsPP and DNA. 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 DNA and suppress the electrostatic repulsion. So, fabrication of the DNA-Au conjugate with DNA directly attached to Au particles has to be carried out at acidic pH.

The most important property of DNA-Au conjugate is its hybridizing specificity. The obtained DNA-Au conjugates were employed to hybridize with their complementary DNA probes, which were immobilized on Au substrates.

AFM assay showed that many DNA-Au conjugates specifically hybridized with its complementary probes on the Au substrates (Fig. 2A). In contrast, almost no gold particle was immobilized when the DNA probes on the gold substrate were not complementary with the DNA-Au conjugates (Fig. 2B).

Figure 2. AFM images of Au-DNA-Au nanostructure. (A) DNA-Au conjugates (DNA1-Au) hybridized with its complementary DNA (DNA2) on a gold substrate. (B) DNA-Au conjugates (DNA1-Au) treated with noncomplementary DNA (DNA1).

CONCLUSION

DNA was directly attached to gold surface to obtain the DNA-Au conjugate for the first time. Acidic pH was found to be indispensable for this conjugate preparation. The obtained DNA-Au conjugate showed high sequence specificity in Au-DNA-Au nanostructure fabrication. The electrochemical characterization of the prepared nanostructure is in progress to obtain more information about DNA as single molecular wire.

REFERENCES