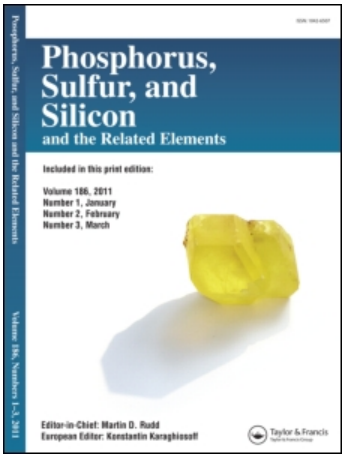

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Chemistry of Oligonucleotide-Gold Nanoparticle Conjugates

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Conjugates prepared by immobilizing thiol-terminated oligonucleotides onto gold nanoparticles form stable colloidal solutions in aqueous media. The oligonucleotides can serve as linkers to organize the gold particles reversibly into three dimensional assemblies, and the gold particles can function as colorimetric reporters for hybridization of the bound oligomers with target oligonucleotides in solution.

Keywords: gold; nanoparticles; oligonucleotide; hybridization; non-radioactive detection

INTRODUCTION

Nanoparticles coated with selected proteins have found applications in immunocytochemistry[1,2] and in clinical diagnostics[3]. Surface modified nanoparticles also show potential as building blocks in the assembly of new materials[4,5]. We summarize here the results of an exploratory study of thiololigonucleotide gold-nanoparticle conjugates. The aims were to see whether the molecular recognition features of oligonucleotides might be exploited in organizing the assembly of nanoparticles, and, conversely, whether the gold nanoparticles might serve as useful reporters for hybridization of oligonucleotides.

RESULTS

Colloidal gold solutions were prepared according to Frens[6] by reduction of tetrachloroauric acid with citrate. Red solutions exhibiting an absorption maximum at 520 nm and a shoulder at 260 nm were obtained. Transmission electron microscopy revealed relatively uniform spherical particles approximately 13 nm in diameter. These data in conjunction with quantitative analysis for gold by atomic emission spectroscopy showed the concentration of nanoparticles to be ~13 nM, with $\epsilon_{520} = 2.4 \times 10^8\ M^{-1}cm^{-1}$ and $\epsilon_{260} = 2.8 \times 10^8\ M^{-1}cm^{-1}$ on a particle basis.

Oligonucleotide-gold conjugates were obtained by treating the gold nanoparticles with synthetic oligonucleotides terminated at the 5'-end with $HS(CH_2)_6OP(O)(O^-)$- or at the

3'-end with $HS(CH_2)_3OP(O)(O^-)$-[7]. Following aging in 0.1 M NaCl, the conjugates were collected by centrifugation, resuspended in the desired buffer-salt solution and filtered. In contrast to solutions of colloids treated with oligonucleotides lacking the thiol group, which turned blue and precipitated in solutions >0.2 M in NaCl, these sols were stable even in 1 M NaCl.

The key reaction in this approach to organizing gold nanoparticles and recognizing DNA sequences is hybridization, a specific association of complementary oligonucleotide strands in which adenine pairs with thymine and guanine pairs with cytosine. We have examined hybridization for a variety of gold conjugates in diverse systems. Some representative examples are provided here. Each is used to illustrate one or more features of the hybridization process; however, the features are common to all the systems. In the notation, "Au" represents a gold nanoparticle and "s-oligonucleotide" represents one of the many thiololigonucleotides linked to that nanoparticle.

Four Component System.

Au-s-TACCGTTG-5' + 5'-AGTCGTTT-s-Au + 5'-ATGGCAACTATACGCGCTAG
I II TARGET 1 ATATGCGCGATCTCAGCAAA-5'

Although the most complex of the self assembly systems thus far investigated, this one was the first examined[8]. The target was a double stranded DNA fragment containing overhanging nucleotide sequences that could bind selectively to different nanoparticle conjugates. In 1.0 M NaCl the mixture afforded a blue solution that reverted to red on heating. The spectral shifts are depicted in Figure 1. Successive heating and cooling experiments monitored by absorbance changes at 260 nm and 700 nm further showed that the transitions are fully reversible. A plausible explanation is that hybridization of the oligonucleotide-gold probes with the target generates a three dimensional network of nanoparticles held in proximity by the oligonucleotide linkers.

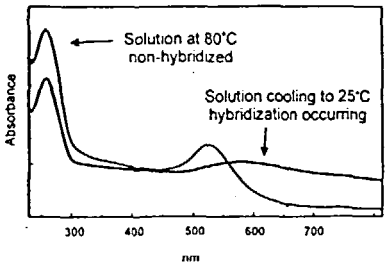

Figure 1. Spectra for probes I + II +Target 1 at 25 °C and 80 °C.

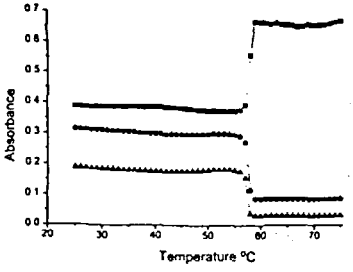

Figure 2. Dissociation curves for V+VI + Target 2 followed at 700 nm (triangles), 620 nm(circles), and 260 nm (squares).

The salient features deduced from this system are that oligonucleotides immobilized on the gold surface can hybridize with complementary oligomers in solution and that hybridization leads to an easily controlled, reversible assembly of the nanoparticles, accompanied by significant shifts in both the visible and ultraviolet spectra.

Two Component System.

Au-s-[SPACER]TCGACCTGGGCAGGGTTC-3' + 3'-AGCTGGACCCGTCCCAAG[SPACER]-sAu
III IV

This system illustrates use of oligonucleotides in assembling nanoparticles with specified connectivity, e.g., formation of assemblies in which III is linked only to IV and IV is linked only to III. Solutions (100 μL) containing III and IV were either allowed to stand at 22 °C for a given period of time or were frozen quickly at -77 °C and allowed to thaw (10 min) at room temperature. Samples (3 μL) were then spotted on a reversed phase TLC plate and dried. The results presented in Table 1 bring out three features. (1) The spotting technique provides a simple and rapid means for observing hybridization involving the gold conjugates; if the nanoparticles have been aggregated by hybridization of the pendant oligonucleotides, the dried spot is blue; if not, the spot is pink. (2) The rate of hybridization increases with the ionic strength of the solution (see data at 22 °C). (3) The rate of hybridization can be greatly accelerated by freezing and thawing the solution. Use of the freeze-thaw cycle in conjunction with the spot tests enables one to effect and detect hybridization within a few minutes.

Table 1. Spot test for hybridization of probes III and IV

Conditions	Freeze-Thaw	Temperature = 22 °C						
Buffer only[a]	Blue	Pink	Pink	Pink	Pink	Pink	Pink	Pink
0.1 M NaCl[b]	Blue	Pink	Pink	Pink	Pink	Pink	Mag.[c]	Blue
0.3 M NaCl[b]	Blue	Pink	Pink	Mag.[c]	Blue	Blue	Blue	Blue
Time (min)	10	0	10	30	60	90	300	720

(a) 10 mM phosphate. (b) In addition to the buffer. (c) Magenta.

Three Component System.

Au-s-[SPACER]-GCGCTAGAGTCGTTT-3' + Au-s-[SPACER]-CCTATCGACCATGCT-3' +
V VI

TARGET 2: 3'-CGCGATCTCTAGCAAAGGATAGCTGGTACGA

This system illustrates use of the oligonucleotide-gold conjugates in recognizing an oligonucleotide target[9]. Dissociation of the assemblies formed by hybridization of V + VI with Target 2 (30 pmoles in 300 μL of solution; 0.1 M NaCl; freeze-thaw procedure) was followed both by spectral changes at three different wavelengths (Figure 2) and by spot tests. In each case the observed transitions were remarkably sharp. For the spot tests the hybridized mixture was warmed in stages (increase of 0.5 °C for each stage, with standing at the new temperature for 5 min before spotting). The color change for the spots occurred within a 1 °C range; at 58.0 °C the spot was blue, at 58.5 °C it was magenta, and at 59 °C

it was pink[9]. We attribute the sharp breaks in the melting transition in these cases to a high degree of cooperativity in dissociating the duplex strands holding the nanoparticle assemblies together and to the fact that the assemblies can exhibit their characteristic spectra even after dissociation of a considerable fraction of the interparticle linkages. As a consequence of the sharp melting transition, the nanoparticle probes are highly selective in recognizing oligonucleotide sequences. Probes V and VI can readily distinguish between Target II and an oligonucleotide differing by a single nucleotide[9]. For a related system we found that a 24-mer target could be identified by the spot test even in presence of four other oligonucleotides, each differing by a single nucleotide substitution, addition, or omission[10].

In summary, oligonucleotides bound through a terminal sulfur atom to gold nanoparticles readily hybridize with other oligonucleotides free in solution or anchored to other nanoparticles. These conjugates provide a means for organizing nanoparticles reversibly into assemblies and show promise as highly selective probes for oligonucleotide targets.

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