NANO LETTERS

2001
Vol. 1, No. 1
32−35

Electrophoretic Isolation of Discrete Au Nanocrystal/DNA Conjugates

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Received October 5, 2000

ABSTRACT

Colloidal nanocrystal/DNA conjugates hold the promise of becoming powerful probes for biological diagnostics as well as versatile building blocks for nanotechnology. To fully realize this potential, it is important to precisely control the number of oligonucleotides bound to the nanocrystal. Here we demonstrate electrophoretic isolation of 5 and 10 nm gold nanocrystals bearing discrete numbers of single-stranded DNA (1–5). The potential use of these discrete conjugates in the fabrication of novel nanostructures is discussed.

Nanocrystal biopolymer conjugates hold great promise both for biological diagnostics, where the nanocrystals can provide unique detection signatures,[1−4] and for nanotechnology, where the information content of the biomolecule can be harnessed for spatial patterning of nanocrystals.[5−11]

There are many strategies available for bioconjugation of nanocrystals, including attachment to biotin−avidin,[1,12] antigen−antibodies,[13] peptides,[14,15] proteins,[15] etc. Among the many biological polymers that can be coupled to nanocrystals, DNA is of particular interest, because of its inherent programmability. The Watson−Crick base pairing of an oligonucleotide is thermally stable at room temperature when the number of bases is around 12. Twelve base pairs are approximately 4 nm in length, or the size of a nanocrystal, and contain a sufficient number of unique pairs that the strand can be designed to contain detailed instructions for placement of the nanoparticle in a programmed assembly. This remarkable property has already been exploited to create three-dimensional aggregates of nanocrystals,[16] to attach nanocrystals to surfaces,[17] and to create small groupings of nanocrystals.[5,6,18] Yet to fully extract the most use from nanocrystal/DNA conjugates, it is first necessary to prepare nanocrystals with a discrete and known number of single-stranded (ss) oligonucleotides attached.

Control over the precise number of oligonucleotides per nanocrystal is essential for diagnostics whenever there is a need to quantify the number of hybridization events, rather than just assess the presence of a particular sequence. Nanocrystals bearing one and only one oligonucleotide strand are particularly important for this purpose. In nanotechnology and three-dimensional construction, we can imagine that nanocrystals bearing different numbers of oligonucleotides could serve as elementary construction units: vertex (4 strands), corners (3 strands), lines (2 strands), terminus (1 strand), etc. Finally, we note that the nature of interaction between the polyanionic oligonucleotides and the nanocrystals is far from known or understood but is a very interesting problem in biophysics. The persistence length of ssDNA is about 2 nm,[19] so in some cases DNA strands may be able to wrap around the particle, and perhaps it is possible to adjust the nanocrystals or the oligonucleotide to promote or suppress this effect. It appears that being able to precisely control the number of bound strands will be very desirable for this purpose.

In this paper we demonstrate the electrophoretic isolation of discrete gold nanocrystal/DNA conjugates. We have chosen Au because of its ease of use—the nanocrystals are stable and processable in the high ionic strength buffers needed for manipulation of DNA, and attachment of DNA to the Au via a terminal thiol on the oligonucleotide is straightforward. In the strategy we are employing, the binding of DNA to the Au is a statistical process. By adjusting the DNA:Au ratio, we can control the average number of DNA strands per particle, but there will always be a distribution of oligonucleotides present. Thus, we need a technique for separation and isolation of conjugates that is very sensitive to the number of bound strands.

Gel electrophoresis is a powerful technique in biology and is widely applied in the separation of DNA of different sizes.[20] In this technique, charged particles migrate in a porous matrix (gel) under an electric field; particle mobility depends on their charge and size. Electrophoresis is also a useful tool in colloidal science, and it was recently applied

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10.1021/nl005508e CCC: $20.00
Published on Web 12/16/2000

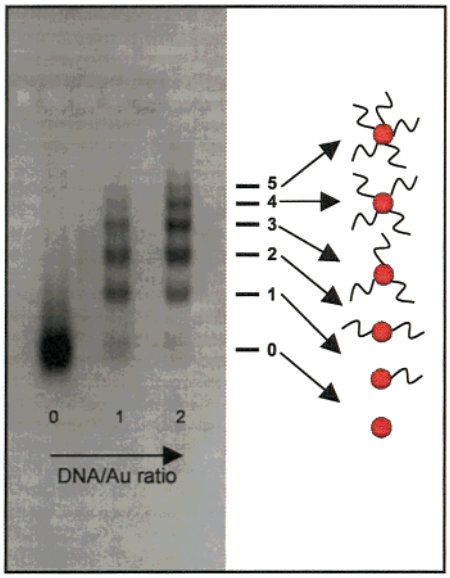

Figure 1. Electrophoretic mobility of 5 nm Au/100b HS-ssDNA conjugates (3% gel). The first lane (left to the right) corresponds to 5 nm particles (single band). When ~1 equiv of DNA is added to the Au particles (second lane), discrete bands appear (namely **0**, **1**, **2**, **3**, ...). When the DNA amount is doubled (third lane), the intensity of the discrete bands change and additional retarded bands appear (**4**, **5**). Because of the discrete character, each band can be directly assigned to a unique number of DNA strands per particle.

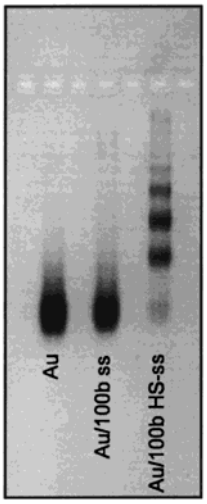

Figure 2. Nonthiolated DNA does not bind to the particles, ruling out nonspecific interaction in these conditions.

to isolate small gold clusters.[21] It seems then a natural choice to use electrophoresis for characterization and isolation of nanocrystal/DNA conjugates since the binding of DNA to nanoparticles should produce a significant shift in their electrophoretic mobility.

We have used Au particles of 5 and 10 nm mean diameter[22] and alkanethiol-modified single-stranded (HS-ss) DNA 18 to 100 bases (b) in length.[23] The conjugates were prepared by addition of the DNA to the Au colloid and were analyzed in 2−3% agarose gels.[24] We first describe the results for 5 nm Au with 100b HS-ssDNA attached, where the most substantial effects are expected. Figure 1 shows the results: discrete bands (**1**, **2**, **3**) of lower mobility appear in the same lane when 100b HS-ssDNA is added to the Au nanoparticles. Doubling the DNA:Au ratio changes the relative intensity of the bands, and in fact new retarded bands (**4**, **5**) appear in the gel. These results clearly indicate a discrete step process that we have assigned as being the number of DNA strands attached to the particles. Each band, corresponding to a defined number of strands per particle, can be recovered from the gel using standard techniques[18] and stored for weeks at 4 °C.

Several experiments have been performed that demonstrate that the discrete bands arise from nanocrystals bearing specific numbers of DNA strands. The issue of greatest concern is that the DNA may interact with Au nanoparticles in different ways (not via terminal thiol). Nonspecific adsorption of DNA lengthwise onto flat Au surfaces is well-known.[25] The DNA could bind nonspecifically to the nanoparticles depending on salt concentration or even promote the clustering of more than one particle. First, the specific binding of DNA through the thiol group was verified. This was done by adding DNA of the same sequence but without the thiol modifier. As it was clearly demonstrated in this control experiment (Figure 2), discrete bands do not appear when 100b ssDNA is used and there is no significant nonspecific binding in these conditions.

Second, it was necessary to prove that the discrete bands do not correspond to nanocrystal clusters (dimers, trimers) mediated by nonspecific DNA interaction. The first experiment consisted in recovering the bands **0**, **1**, **2**, and **3** (see Figure 1) and imaging the particles in a transmission electron microscope (TEM).[26] For the four bands collected, there was no difference in the particle distribution on the TEM grid. When these samples were run again in the gel, their mobility remained unchanged. Unless there is some unexpected strong interaction with the carbon substrate on the TEM grid, the change in mobility cannot be assigned to nanocrystal cluster formation.

The second experiment involved mixing the recovered conjugate bands with free particles and then, after several hours, running the mixture through a gel. Again, if the discrete bands corresponded to nanocrystal clustering due to some interaction with DNA, adding free particles should disrupt this equilibrium and induce the reappearance of the other bands. Figure 3a shows that in all cases the lanes corresponded to a superposition of the original conjugate and free particle bands. In addition, this experiment also proves that the HS-ssDNA is strongly bound to the Au, since there is no redistribution of number of DNA strands per particle.

An additional experiment was to study particles of different sizes. Figure 3b shows the bands for 5 nm Au/100b HS-ssDNA and 10 nm Au/100b HS-ssDNA conjugates. Because of the sieving effect of the gel, 10 nm particles migrate less than 5 nm particles, and the shift of 10 nm Au/100b HS-ssDNA conjugates is smaller than the 5 nm Au/100b HS-ssDNA ones. When 5 and 10 nm particles are mixed with subsequent addition of DNA, it should produce a superposition of the two sets of bands. In contrast, if particle clusters were formed all combinations would be possible (5−5, 5−10, and 10−10 nm dimers; 5−5−5, 5−5−10, and other

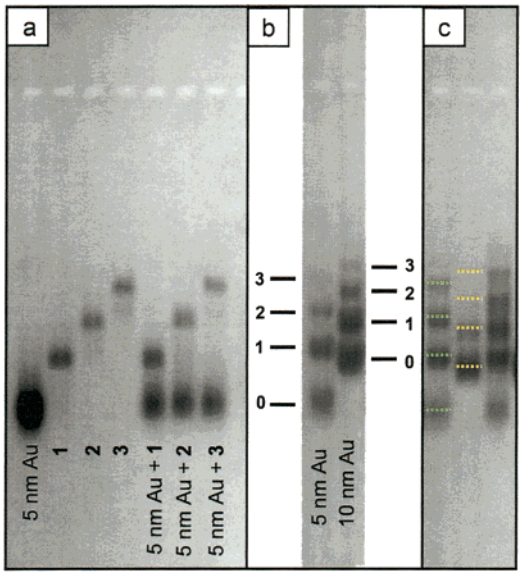

Figure 3. (a) 5 nm Au sample and recovered 5 nm Au/100b HS-ssDNA conjugate bands (**1**, **2**, **3**), showing the stability of the conjugates. Mixing each recovered band with an equivalent amount of 5 nm Au colloid (Au + **1**, Au + **2**, Au + **3**) generates superposed bands; this result clearly demonstrates that the bands are not due to nanoparticle clustering (dimers, trimers, etc.). (b) Au/100b HS-ssDNA conjugates for 5 and 10 nm particles. (c) Adding DNA to a mixed solution of 5 and 10 nm particles (last lane) leads to a superposition of 5 nm Au/100b HS-ssDNA (first lane) and 10 nm Au/100b HS-ssDNA (second lane) bands, corroborating the nature of the Au/DNA conjugates.

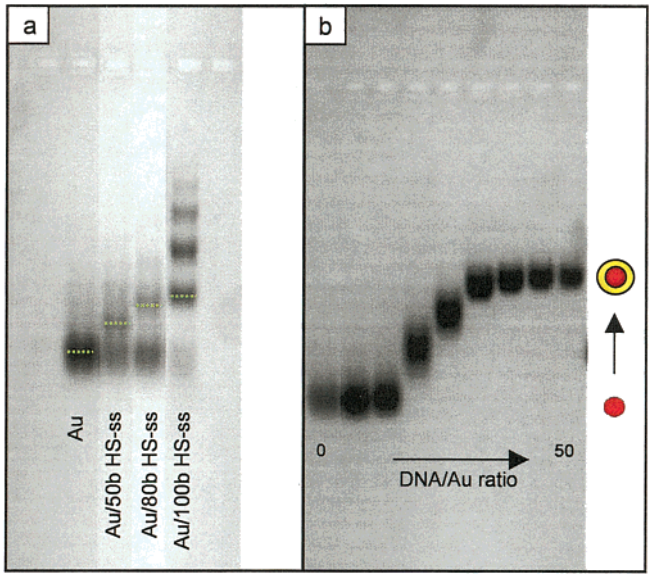

Figure 4. (a) 5 nm Au sample and first Au/DNA conjugates (···) of different DNA lengths (50b, 80b, and 100b; 3% gel). (b) Electrophoretic mobility of 5 nm Au/18b HS-ssDNA (2% gel). The DNA:Au molar ratios from the left to the right are 0 (pure colloid), 1, 2, 5, 10, 20, 30, 40 and 50; these values reflect the increase of the number of DNA strands per particles, but they do not correspond to the actual values.

trimers; etc.), and a much larger number of bands would be superposed, probably resulting in a single spread band. In Figure 3c, the first two lanes correspond to the control samples of 5 and 10 nm particles, prepared in the same conditions as in the mixture (Au concentration and Au:DNA ratio), while the third lane corresponds to the mixed solution. It is clear that the third lane keeps the discrete character and that all the bands can be assigned to either 5 nm Au/100b HS-ssDNA or 10 nm Au/100b HS-ssDNA conjugates. The mobility pattern also suggests that the DNA preferentially binds to the 10 nm particles when both 5 and 10 nm are present in the same solution, but further experiments need to be done to understand this effect.

The success of the electrophoretic isolation of nanocrystal/DNA conjugates depends on several factors. First, the nanoparticle sample must be homogeneous in charge and particle size distributions in order to obtain narrow bands. These two factors can be monitored and in some ways regulated. In the case of homogeneous samples, the limitation of the electrophoresis becomes the intrinsic mobility of the particles and DNA. The mobility of 100b HS-ssDNA and 5 nm particles is similar in our conditions so the conjugation of the two leads to a significant shift in their mobility. For shorter DNA strands, which migrate faster in the gel, the effect on the particle mobility should be smaller. Figure 4a emphasizes the difference in the mobility of the first nanocrystal/DNA conjugate (one strand) for 50b, 80b, and 100b HS-ss DNAs. We note that for DNA sizes below 50b, the **0** and **1** bands will be barely resolved in these conditions and it will be difficult to isolate the discrete nanocrystal/DNA conjugates. Increasing the gel concentration (increased resolution) or running time will not improve significantly the separation due to the spread of both bands and, in this case, a better nanoparticle size/charge distribution is probably required. Nonetheless, we could still use electrophoresis for short ssDNA conjugates, for example, by hybridizing the short strand with a long one, then isolating the conjugates and finally releasing the long ssDNA.

Gel electrophoresis can be also exploited to determine the average number of strands per particle, for these short DNA strands. Figure 4b shows the results for 5 nm Au/18b HS-ssDNA conjugates. The first lane corresponds to the 5 nm unconjugated nanoparticles, and the second and the third lanes correspond to samples prepared with DNA:Au molar ratios of 1 and 2. While the particles run in well-defined bands, the addition of small amounts of 18b HS-ssDNA does not affect the overall mobility of the particles. Nevertheless, by increasing the DNA:Au ratio to 5 or 10, multiple strands attach to the particles, producing a decrease in the particle mobility. We achieve a saturation limit around 20 DNA:Au molar ratio, above which the particle mobility does not change significantly. However, we note that the later bands are narrower, which indicates more homogeneous charge due to a better coverage with DNA. Decreasing the gel concentration would help distinguish the bands above 20. Unfortunately we cannot directly assign the DNA:Au molar ratio in solution with the actual number of DNA strands per particle. We can, however, infer that in lane 5 there is more DNA per particle than in lanes 2 and 3. Methods to quantify the actual average number of DNA strands per particle are being developed; this will be a subject of future studies.

In summary, we have been able to isolate discrete nanocrystal/DNA conjugates by gel electrophoresis. Using

5 and 10 nm Au nanoparticles, we demonstrated that the shift in the particle mobility due to the DNA attachment is a very powerful probe used to study and isolate several conjugates; this method can also be extended to different systems. Particles with a well-defined number of DNA strands can be seen as new building blocks in nanotechnology as well as important probes for quantitative detection analysis.

Acknowledgment. D.Z. is grateful to FAPESP, proc. 99/08603-7, for financial support and to the Brazilian National Synchrotron Laboratory (LNLS) for travel expenses. C.M.M. is a Howard Hughes Medical Institute Predoctoral Fellow. W.J.P. was supported by the German Research Foundation (DFG). We thank the National Center of Electron Microscopy at Lawrence Berkeley National Laboratory and the Electron Microscope Laboratory at LNLS for the use of TEMs. This work was supported by NIH National Center for Research Resources, Grant 1 R01 RR-14891-01 through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098, DOD Advanced Research Projects Agency (DARPA) under Grant No. N00014-99-1-0728 and (in part) by the Director, Office of Energy Research, Office of Science, Division of Materials Sciences, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. The views expressed in this paper are not endorsed by the sponsor.

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(22) Gold colloids were either purchase from Ted Pella (Redding, CA) or synthesized by the citrate/tannic acid method (Handley, D. A. In *Colloidal gold: principles, methods and applications*; Hayat, M. A., Ed.; Academic Press: New York, 1989; Vol. 1, pp 13−32). Typical size distributions were 15%. The stability of gold colloids was increased by complexation with bis(*p*-sulphonatophenyl)phenylphosphine dihydrate, dipotassium salt (Strem Chemicals, Newbutyport, MA).[18] The modified colloids were precipitated with NaCl, centrifuged, and redissolved to a final concentration of 1−10 μM.
(23) Custom DNA were purchased from Integrated DNA Technologies (Coralville, IA). DNA sequences: 100b, 5′-XGCAGTAACGCTATGTGACCGAGAAGGATTCGCATTTGTAGTCTTGAGCCCGCACGAAACCTGGACACCCCTAAGCAACTCCGTA, CAGATGGGAACAGCA-3′; 80b: 5′-XGCAGTAACGCTATGTGACCGAGAAGGATTCGCATTTGTAGTCTTGAGCCCGCACGAAACCTGGACACCCCTAAGCAACTC-3′; 50b, 5′-XGCAGTAACGCTATGTGACCGAGAAGGATTCGCATTTGTAGTCTTGAGCCC-3′; 18b, 5′-XCAGTCAGGCAGTCAGTCA-3′. X = 5′-thiol modifier.
(24) Nanocrystal/DNA conjugates were prepared by adding a stoichiometric amount of 50 μM DNA (1, 2, etc. equiv) to the gold colloid in the 0.5X TBE (Tris−borate EDTA buffer), vortexing for a few seconds and adding NaCl up to 50 mM, vortexing again, and incubating the solution at room temperature for 2 h before running the gel. The electrophoresis experiments were performed in 2 or 3% agarose gels at 100 V, 6.7 V/cm, 1 h. We used 0.5X TBE as running buffer. Gold colloids in this size range have a deep red color and can be easily visualized in the gels.
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(26) TEM was performed on a Topcon EM002B electron microscope (200 kV) at NCEM, Lawrence National Laboratory (Berkeley, CA) and on a JEM-3010 (300 kV), LME- LNLS (Campinas, Brazil).

NL005508E