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Haptenylated mercaptodextran-coated gold nanoparticles for biomolecular assays

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Gold nanoparticles coated with haptenylated mercaptodextrans bind specifically to paramagnetic beads coated with the corresponding antibody

Recent developments in analytical nanotechnology have suggested that it may be possible to replace conventional labels with nanoparticles,[1] but it is important to remember that the quality of the label is only one of several factors that contribute to the sensitivity and reliability of an analytical method. The sensitivity depends on the minimum signal that can be distinguished from the background; factors that increase the background will reduce the sensitivity. In biomolecular assays a major source of background is non-specific binding. An example would be the binding of antibodies to nanoparticle conjugates through hydrophobic interactions, primary amines and mercapto-groups, rather than by specific interaction with the corresponding antigen. Previous experience has shown that this problem can be avoided by using blocking agents, but it is important to ensure that these do not displace the molecules that have been conjugated to the nanoparticles. One of the most exciting opportunities to have emerged from the integration of nanotechnology and analytical chemistry, is the possibility of fine tuning the optical properties of reporter particles so that more than one analyte can be determined in the same sample. An example is the use of different diameter gold nanoparticles to distinguish between DNA from different sources,[2] but if these methods are to yield reliable results it is important to ensure that conjugated molecules are unable to transfer from one particle to another.

The attachment of molecules to metallic nanoparticles is a dynamic process in which bound molecules are in equilibrium with unbound molecules in solution. If the molecules in solution are removed, some bound molecules will be detached and the equilibrium will be restored; this is why mercapto compounds are detached from washed gold nanoparticles.[3] Molecules that are not bound tightly are displaced by molecules that do bind tightly, as shown by the displacement of aminodextrans from silver nanoparticles by tris(3-mercaptopropyl)-*N*-glycylaminomethane.[4] When two compounds with the same functional groups compete for a limited number of particles, the compound that is present at the highest concentration will displace the other, as shown by place exchange studies.[5] When two compounds are present at the same functional group concentration, the compound with the most functional groups per molecule will displace the other. These concepts are important because biomolecular assays are often carried out in the presence of proteins and other molecules that can compete with conjugated molecules. One way to ensure that conjugated molecules are not displaced is to attach them to a polymeric shell from which the particles cannot escape.[6] An alternative approach is to conjugate them so firmly that they remain bound in the presence of any competing molecule. For metal nanoparticles the latter approach implies that the molecules should be bound by mercapto groups, and that the number of these groups should exceed the number presented by any potential competitor. In this communication the conjugation of haptens to gold nanoparticles with dextrans containing 15 mercapto groups is described.

Once a molecule has been conjugated to a nanoparticle it must still be able to interact with complementary molecules in solution. In aqueous solution a shell of water molecules that repel each other surrounds most biological molecules. This shell can be used to curtail non-specific binding, but before two complementary molecules can interact it must be pierced; this can be facilitated by allowing one of the molecules to protrude beyond the shell.[7] When complex biological molecules such as antibodies and antigens interact they undergo a series of conformation changes as they form a more stable complex. If these changes are restricted the complex will take longer to form and be less tightly bound.[7] From these considerations it follows that conjugated molecules should protrude from a flexible hydrophilic surface. These conditions are satisfied by the haptenylated mercaptodextrans described in this communication; the dextran backbone is flexible and hydrophilic,[8] and the target molecules are located at the end of hydrophobic projections.

Gold nanoparticles (12 nm diameter) were prepared by citrate reduction of $HAuCl_4$ at ebullition. Excess citric acid was removed by dialysis before coating with mercaptodextrans. Antibodies (Anti-DNP; Sigma) were biotinylated with biotinamidocaproate *N*-hydroxysuccinimide ester, and slow tilt rotated with streptavidin coated paramagnetic beads (Dynal). Antibody coated beads were separated from unbound antibodies by magnetic precipitation and resuspension in PBS. By titrating a fixed amount of aminodextran with different amounts of 6(2,4-dinitrophenylamino)-1-aminohexanoic acid [*N*-hydroxysuccinimide ester][9] it was possible to prepare dextrans that were only substituted with one 2,4-dinitrophenyl (DNP) hapten per molecule. The remaining primary amines were reacted with 3-(2-pyridyldithio)propionic acid HNS ester;[10] before mixing with the nanoparticles the 2-pyridyldisulfide structures were converted to free mercapto groups by reduction with dithiothreitol. The structure of a haptenylated mercaptodextran molecule is shown in Fig. 1. The DNP is linked to the dextran by a hydrophobic linker of a kind that is known to enhance

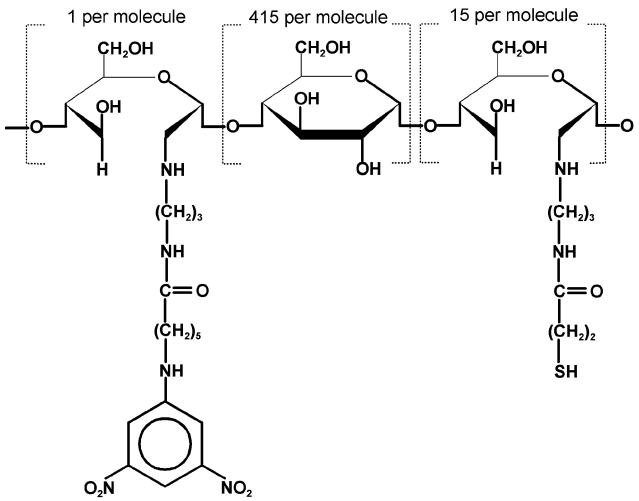

Fig. 1 Haptenylated mercaptodextran molecule.

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antibody affinity,[11] and the number of mercapto groups per molecule is an order of magnitude higher than the number likely to be encountered in biomolecular assays. By mixing the nanoparticles with different amounts of dextran it was established that a minimum of four dextran molecules per particle was required to prevent flocculation when PBS was added; particles coated with this minimum amount of dextran was used in all subsequent experiments. The absorbance spectrum of the dextran coated particles was identical to spectrum of citrate stabilized particles suggesting that the conjugates were not interconnected, but further work is required to confirm this. Before investigating the conjugates with antibodies BSA and Tween-20 were added to curtail non-specific binding. After standing for one week the conjugate solution was slow tilt rotated with antibody coated paramagnetic beads. After rotating for ten minutes the beads were magnetically precipitated and the UV/vis spectrum of the supernatant was recorded. Fig. 2 shows how the absorbance spectrum changed as the amount of beads increased; no change was observed in control experiments when rotation was carried out in the presence of 10 μM DNP, or when beads coated with non-specific (anti-mouse) antibodies were used. When magnetically precipitated antibody coated beads were resuspended and rotated with 10 μM DNP, bound nanoparticles were released into solution. These results show that gold nanoparticles coated with haptenylated mercaptodextran bind specifically to the corresponding antibody. Results were unaffected by BSA and Tween-20 showing that the haptenylated mercaptodextrans were not displaced. This method of conjugation is suitable for most haptens, and other molecules, including oligonucleotides and antibodies. Although it was possible to detect picomolar amounts of antibody (Fig. 3) relatively large amounts of expensive paramagnetic beads were consumed. This could be avoided by determining the amount of nanoparticles bound to the beads rather than the concentration remaining in the supernatant. A suitable platform for such determinations would be a dark field microscope, which can detect sub-femtomolar concentrations of particles.[12]

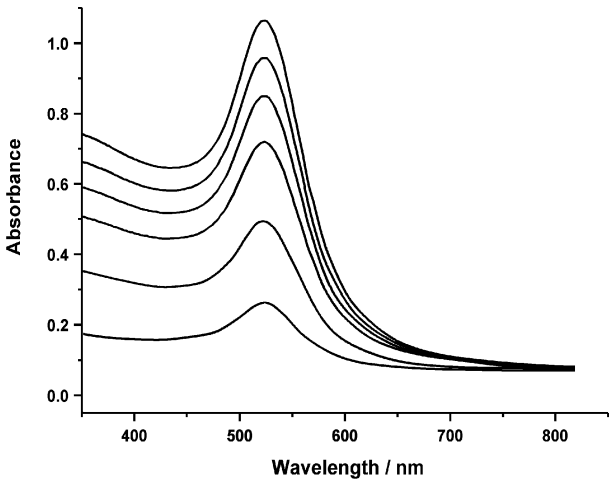

Fig. 2 Decrease in absorbance of conjugate solution as the amount of antibodies bound to paramagnetic beads was increased.

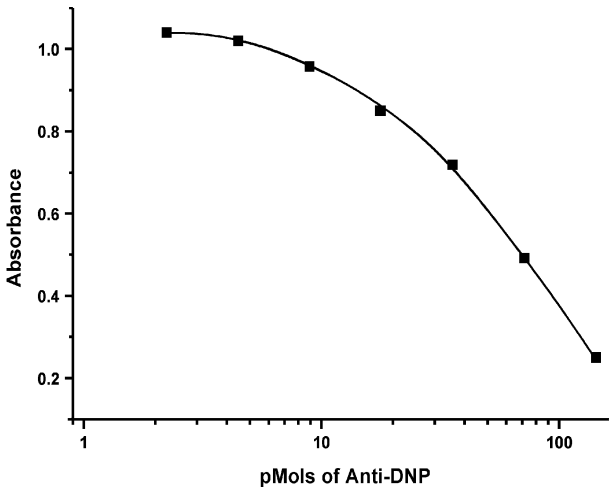

Fig. 3 Plot of decrease in absorbance of conjugate solution as the amount of antibodies attached to paramagnetic beads was increased.

Notes and references

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