Highlights

Detection in Immunoassays

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Immunoassay is an important analytical method that utilizes the high selectivity afforded by antibody molecules and is used in various fields, including basic life science studies and medical diagnostics. The immunoassay process consists of molecular recognition by antibodies or aptamers and detection. Improvements of the detection part are necessary for higher sensitivity of analysis. The most widely used immunoassay format is the enzyme-linked immunosorbent assay (ELISA), which uses amplification by the enzymatic reaction for detection. In many cases, the choice of the enzyme reaction products with higher extinction coefficients is desirable, since quantification is mainly performed by measuring the absorbance of the products. In recent years, the choice of nanoparticles instead of small compounds as a product has been reported.

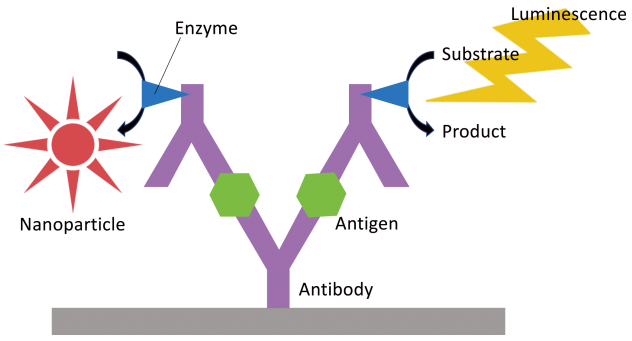
The uses of plasmonic nanoparticles, including gold nanoparticles (AuNPs) as substrates, or products of the enzyme reaction in ELISA, allows for improvements of the sensitivity because of extremely high extinction coefficients of the plasmonic AuNPs. Some enzymes, including catalase, glucose oxidase, and alcohol dehydrogenase, have been used to catalyze the growth of metal nanoparticles to realize sensitive colorimetric detection.[1-3] In addition, a nanoparticle-based immunoassay method that does not use enzymatic reactions have also been reported. The method is based on spectral changes of the visible absorption when antigens bind to antibody-labeled gold nanoparticles.[4]

Nanoparticles can also be used for fluorescence detection. For example, up-converting nanoparticles were labeled with antibodies and used for immunochromatography.[5] In addition, it was also suggested that the sensitivity of conventional fluorescence immunoassays may be increased by using a silver nanoparticle-immobilized well plate due to its fluorescence enhancement effect.[6]

Some nanomaterials are used as nanocarriers that can release large amounts of signal reporters. For example, an antibody and hundreds of DNA barcodes were conjugated onto the same AuNP, and then the capture of a target protein was translated into the detection of large numbers of DNA barcodes.[7] In another study, in the presence of the target, a single liposome involving large amounts of cysteine molecules was immobilized through an antigen-antibody complex, and then ruptured to release cysteines, which could induce the rapid aggregation of AuNPs.[8] Moreover, the sensitivity of ELISA can also be improved by loading large numbers of enzymes onto a single AuNP.[9] Furthermore, protein nanoparticles containing hydrophobic polypeptides and antibody binding domains were used for an immunoassay by incorporating large numbers of hydrophobic fluorescent molecules into the protein nanoparticles.[10]

Another technique for sensitive detection in immunoassays is chemiluminescence. For example, chemiluminescent competitive indirect ELISA with a pretreatment with immunomagnetic beads was reported.[11] Whereas peroxidase-luminol-based chemiluminescence is a popular method for chemiluminescence immunoassay, a bioluminescent immunoassay using Cypridina luciferase has also been reported.[12] In addition, a nanomaterials-based system has been developed to improve the sensitivity of chemiluminescence. Silver, gold, and platinum nanoparticles have strong catalytic properties for chemiluminescence reactions because of an increased surface area and surface electron density.[13-15]

The immunoassay method is expected to become a more sensitive and easy-to-use analytical tool by combining immunoassays with portable detectors, microfluidic devices, or a lateral flow format (immunochromatography) in addition to further improvements in the detection sensitivity.



Detection with nanoparticles and by chemiluminescence in immunoassays.

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