Development of an Immunosensor of Aflatoxin B1 Based on Silica-coated Gold Nanoparticles

William Wu

Princeton International School of Mathematics and Science

Abstract

Due to the common presence of AFB1 in human diets and its toxicity, sensitive and cheap detection methods of AFB1 are needed. Many detection methods, including TLC, HPLC, ELISA, exist, but the most commonly accepted and commercially available method utilizes the intermolecular interactions between gold nanoparticles and AFB1 antibodies. However, such interactions are often weak, and gold nanoparticles tend to aggregate on their own. Thus, by coating gold nanoparticles with a layer of silica, the thermodynamic stability of the antibody-gold nanoparticle bioconjugate can be increased. Moreover, the hydroxyl groups on the outer layer of the silica coating can connect the antibody with gold nanoparticles through strong chemical bonds. In this project, gold nanoparticles will be synthesized by using the citrate method and coated with a silica layer by using previously reported methods. Different methods of connecting the antibody and gold nanoparticle will be evaluated. More specifically, these methods include using reductive amination, michael addition, and esterification.

Introduction

Aflatoxins are toxic metabolic products produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which are two fungus commonly found in grain storage facilities and spoiled corn food (*1*). Aflatoxins is a class 1 carcinogen with a TD50 of 3.2 μg/kg/day in rats (*1*). Among all the toxins in this group, Aflatoxin(AFB1) is one of the most common contaminants in human's daily diet, such as peanut, corn, and other grains (*1*). AFB1 is also considered as the most dangerous and poisonous toxin in the group (*1*).

Antibody-gold nanoparticles bioconjugates have been widely used as biosensors (*2*). This is because of gold nanoparticles' ease of synthesise, functionalization, and biocompatibility (*2*). Antibodies are usually connected to gold nanoparticles via chemisorption methods and physisorption methods (*2*). In chemisorption, the antibody or gold nanoparticles are often modified in certain ways so that a chemical bond can be formed between them (*2*). This method ensures the strong connection between gold nanoparticles and antibodies (*2*). Moreover, it can often make sure that the active site of the antibodies will not be connected to the gold nanoparticle, which will make the sensor dysfunctional (*2*). In physisorption, the antibodies are connected to the gold nanoparticles via electrostatic, hydrophobic interactions, and van der Waals forces (*2*). This method is easy to use as the antibody and gold nanoparticles do not need to be modified (*2*). However, the connection between them is relatively weak and a correct orientation of the antibody can not be guaranteed (*2*).

AFB1 antibodies secreted by the B cells of mammals can be classified as immunoglobulin G antibodies (*3*). Immunoglobulin G is one of the most common types of

antibody found in human blood circulation (*4*). Each immunoglobulin G antibody has carbohydrate moieties, two antibody binding sites connected with disulfide bonds(figure 1) (*2*).

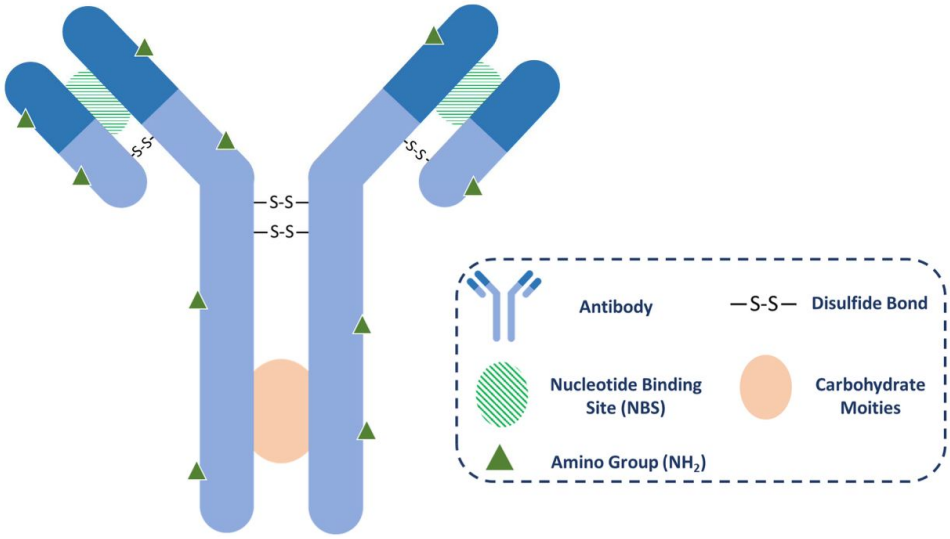

Fig. 1. Structure of Immunoglobulin G Antibody

In this project, AFB1 antibodies are connected to silica-coated gold nanoparticles via chemisorption methods. Compared with classical antibody gold nanoparticle bioconjugate, this novel bioconjugate should exhibit higher thermodynamic stability and sensitivity and consistency due to stronger connections between antibodies and nanoparticles.

Methods

I. Synthesis of silica-coated gold nanoparticles

Gold nanoparticles will be synthesized by reducing Au^{3+} ions with sodium citrate in water at 80 °C (*5*). Silica coating will be installed on the gold nanoparticles by first modifying the surface of gold nanoparticles with 3-aminopropyl-trimethoxysilane (APMS) (*5*). TEOS and NaOH will be subsequently added to the solution to initiate polymerization in the silica layer (*5*).

II. Synthesis of antibody silica-coated gold nanoparticle bioconjugates

AFB1 antibody will be obtained commercially.

a. Reductive amination method

The hydroxyl groups in the carbohydrate moieties of the AFB1 antibody can be mildly oxidized into carbonyl groups with $NaIO_4$, which can then be modified into an imine by adding a primary amine. The imine can be reduced by using $NaCNBH_3$ (*2*).

Chloro(2-chloroethyl)dimethylsilane(figure 2), a commercially available molecule, will be used as the starting material. Ammonia will be added to the solution to substitute the chlorine connected to carbon with $-NH_2$, which can then form an imine with the antibody, and the hydroxyl group on the outer layer of the silica coated gold nanoparticles can connect to the silicon atom.

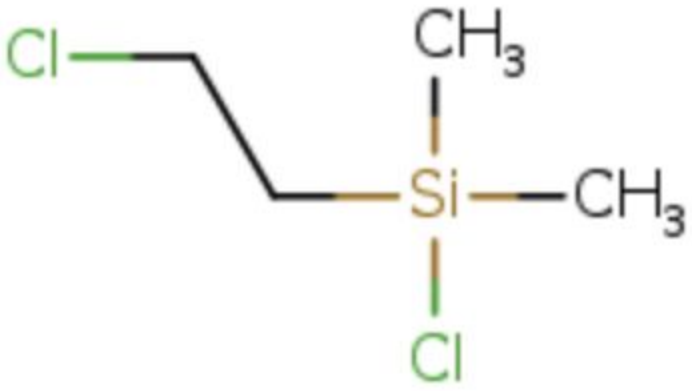

Fig. 2. Chloro(2-chloroethyl)dimethylsilane

b. Michael addition

Acryloyl chloride(figure 3) will be added to the solution of silica coated gold nanoparticles. Esterification reaction is expected to occur between the hydroxyl groups on the

outer layer of the silica coated gold nanoparticles and acryloyl chloride. Primary amine groups can undergo michael addition reaction with the newly functionalized gold nanoparticle complex. Similar reactions to connect the immunoglobulin G antibody with gold nanoparticles have been reported before (*2*).

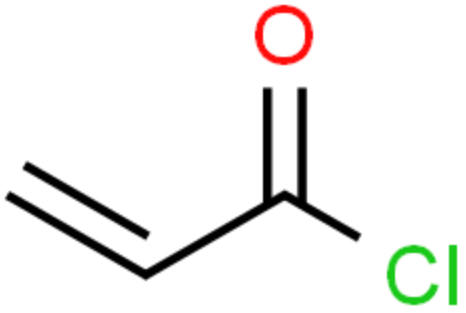

Fig. 3. Acryloyl chloride

c. Esterification method

Succinyl chloride(figure 4) will act as the anchor between the antibody and gold nanoparticles. More specifically, due to the presence of two acyl chloride functional groups, the molecule can form an amide with the amine groups on the antibody and a ester with the hydroxyl groups on the gold nanoparticles.

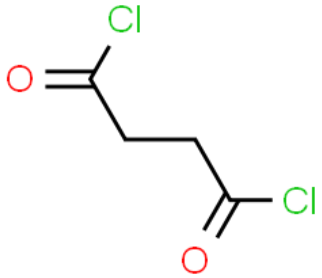

Fig. 4. Succinyl chloride

III. Construction of sensor

A sensor based on liquid chromatography will be constructed based on previously reported methods. The structure of the sensor is shown in figure 5 (*2*).

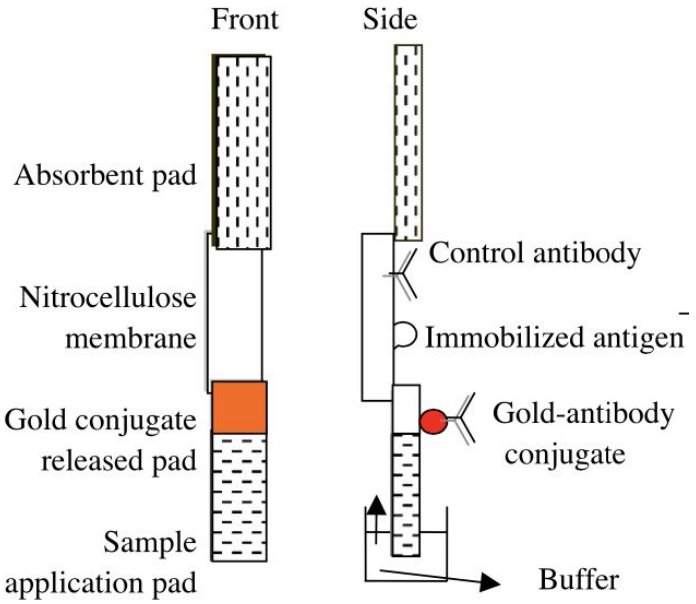

Figure. 5. Structure of the sensor

IV. Evaluation of the sensor

Dilute solution of AFB1 toxin(1ng/ml), obtained commercially, will be made in the fume hood with gloves and goggles and under adult supervision. The sensor will be tested by dipping it into the solution and waiting for color change. AFB1 toxin solution will be handled with great care. Aflatoxin B1 will be treated with 6% solution of hydrogen peroxide at pH 9.5 for 30 minutes at 80 °C to detoxify the toxin (*6*).

Reference

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