Synthesis and characterization of anti-body self-assembling monolayers on the surface of a quartz crystal microbalance for use as a biosensor
by Napawan Kositruangchai

A thesis submitted in partial fulfillment Of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
A biosensor that could detect biological pathogens in contaminated drinking water would be very useful for preventing illnesses caused by these pathogens. The objective -of this work has been to bind anti-bodies for a bacterial pathogen to a Quartz Crystal Microbalance (QCM) in order, to make such a biosensor. A QCM is one of the most popular devices that can be used as a transducer for a biosensor because it is very small, inexpensive, portable, rapid, and has utility in a flow cell, and minimal plectrical and electronic requirements. The QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. This crystal will oscillate with changing frequency when the surface changes in an alternating electric potential. When the mass is increased, it will decrease the oscillation frequency so when molecules adsorb to the QCM surface, the frequency in the output signal will decrease.

In order to make an effective biosensor, a self-assembled monolayer was grown on the QCM surface. This SAM contains a thiol-terminated acid having an amide bond with an adipic dihydrazide terminus. The dihydrazide is then reacted with antibody to covalently bond the antibody to a gold surface.

X-ray photoelecton spectroscopy (XPS) and angle dependent XPS (20,40, 60 and 80 degrees) have been used to confirm the binding of Au-S in alkane and acid thiol, and the presence of amide group in adipic dihydrazide and antibody. XPS not only confirmed the binding to the surface, but also has been used to observe thickness and orientation of layers. In addition, hydrated and dehydrated QCM were observed for the orientation of antibodies.

XPS analysis, elution with tween20, and performance of the QCM sensor tests all confirm that the antibody is covalently bound to the acid thiol surface but not to the alkane thiol surface. The QCM, which were reacted with acid thiol, dihydrazide and antibody, showed a significant drop in frequency when exposed to solution containing the antigen. The results indicated that the method developed is effective for making a biosensor capable of detecting bacterial toxins in water.

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APPROVAL

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Napawan Kositruangchai

This thesis has been read by each member of the thesis committee, has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Approved for the Department of Chemical Engineering

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ABSTRACT

A biosensor that could detect biological pathogens in contaminated drinking water would be very useful for preventing illnesses caused by these pathogens. The objective of this work has been to bind anti-bodies for a bacterial pathogen to a Quartz Crystal Microbalance (QCM) in order to make such a biosensor. A QCM is one of the most popular devices that can be used as a transducer for a biosensor because it is very small, inexpensive, portable, rapid, and has utility in a flow cell, and minimal electrical and electronic requirements. The QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. This crystal will oscillate with changing frequency when the surface changes in an alternating electric potential. When the mass is increased, it will decrease the oscillation frequency so when molecules adsorb to the QCM surface, the frequency in the output signal will decrease.

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CHAPTER 1

INTRODUCTION

Motivation and Objectives

In the environment, there are a multitude of diseases that are transmitted by contaminated water. These diseases can cause illness in many people. Food-borne and water-borne enteric pathogens[1] are a main cause of illness in the US and in the world. Devices used to measure for microbial contaminants are difficult to use and normally unavailable. This thesis is part of an ongoing project to develop a portable rapid biosensor that could be used for on-site detection of water-borne and food-borne microbial pathogens in water.

A biosensor is a tool that can measure concentrations of biologically important chemicals and also uses a biological material to detect the analyte [2]. Typically, a biosensor consists of two parts: a receptor and a transducer. The receptor selectively reacts with a particular analyte of interest. The transducer can convert the interaction of the receptor and the analyte to a quantifiable signal.

The biosensor considered in this work is based on a Quartz Crystal Microbalance (QCM). The QCM is one of the most popular tools in mass-sensitive detection [3] that can be used as a transducer for biosensors. The advantages of the QCM are that it is very small, inexpensive, portable, rapid, efficient, has minimal electrical and electronic

requirement, can be used in a flow cell, and provides for sequential refinement of positive signals. QCM is one type of acoustic wave sensor that can propagate in a specially cut crystal. The crystal will oscillate when the surface changes under an alternating electric potential with changing frequency. When the mass is increased, the crystal will decrease the oscillation frequency as described in the Sauerberey equation (equation 1) [4].

$$\Delta f = -\frac{f_R}{\rho_Q dA}\Delta m = -\frac{f_R^2}{NA}\Delta m \qquad (1)$$

Where Δf is the frequency change, f_R is the resonant frequency, ρ_Q is the density of the crystal, d is the thickness of the crystal, A is the area of the electrodes, Δm is the mass change, and N is the frequency constant of the quartz.

In order to make the QCM an effective biosensor, the QCM has to be coated with suitable capture agents. These capture agents must have a high degree of specificity and affinity for the targets [1]. After they are linked to the QCM, these capture agents must remain stable and tightly associated with the electrode during sample analysis.

The objective of this thesis has been to couple an antibody to the gold surface of a QCM so that it can be used as a receptor for the sensor. For this experiment, Anti-LT (anti-*Escherichia coli* heat – labile enterotoxin) gift of W. Ceiplak (Corixa Corp.) was used as a capture agent. For the improved biosensor, we have used a method for organizing a derivatized self-assembled monolayer (SAM) on the gold surface of the QCM. The SAM contains a thiol-terminated alkane having an amide bonded with adipic dihydrazide terminus. This dihydrazide is then reacted with the antibody to covalently bond the antibody to the gold surface [1]. The system of SAM is shown in the cartoon of

Figure 1. XPS (x-ray photoelectron spectroscopy) and angle dependent XPS were used to examine the surface.

The objectives of this experiment were to: (i) optimize reaction conditions to obtain high density and reactivity of the antibody on the surface, (ii) characterize the thickness and orientation of antibody layer, and (iii) determine whether antibody is covalently bound to the adipic dihydrazide.

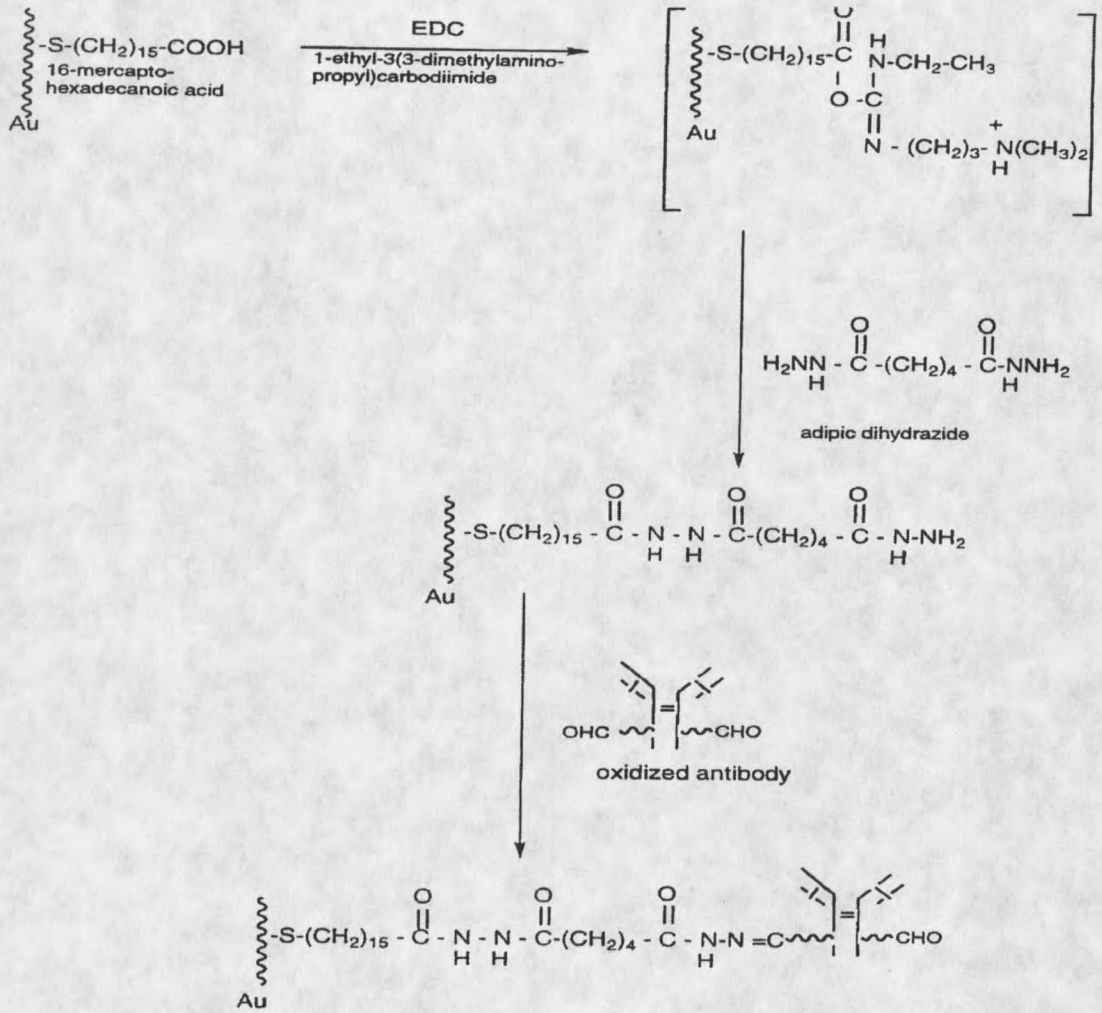

Figure 1. Reaction scheme for the functionalization and coupling of antibody to a self-assembled monolayer on a gold electrode surface. The antibody was oxidized with sodium periodate prior to addition.

CHAPTER 2

BACKGROUND

Antibodies

Antibodies are host proteins produced in reaction to the antigens that are the foreign molecules in the body [5]. Antibodies are produced by plasma cells and the precursor-B-lymphocytes, then circulate through the body blood and lymph. An antibody binds specifically with an antigen. Then the antigen-antibody complexes are removed from circulation by macrophages through phagocytosis. Because of this specific antibody-antigen binding, antibodies are very important reagents to use in immunological research and clinical diagnostics.

Structure of an Antibody (Immunoglobulin) Molecule

Antibody (or immunoglobulin) molecules are glycoproteins composed of one or more units, each unit containing four polypeptide chains [5] two identical light chains (L) and the other two identical heavy chains (H) in Figure 2. The difference of the amino-terminal end of each polypeptide chain shows variation in amino acid composition and is referred to as the variable (V) regions. The light chain (L) consists of a variable domain (V_L) and a constant domain (C_L), and the heavy chain (H) consists of a variable domain (V_H) and three constant domains (C_H^1, C_H^2, and C_H^3). The amount of amino

acids, along with the molecular weight of each heavy chain, is approximately twice that of each light chain. The chains are bound by a combination of noncovalent interactions and in most antibodies, by covalent interchain disulfide bonds that form a bilaterally symmetric structure. The V regions of H and L chains include the antigen

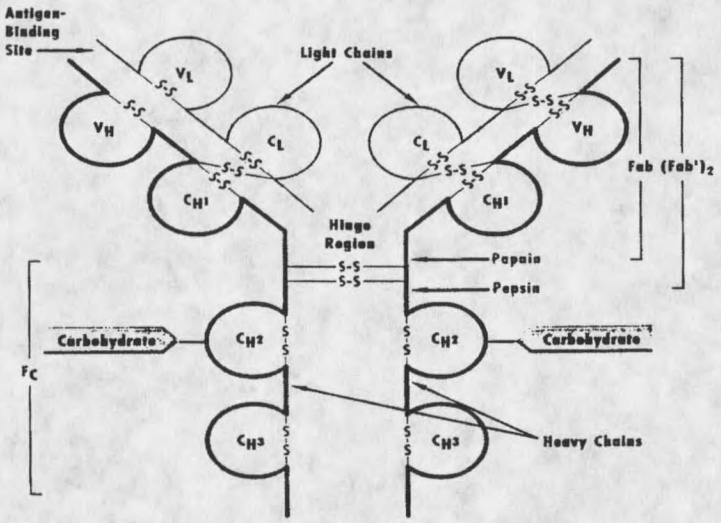

Figure 2. The antibody molecule.

binding sites of the immunoglobulin (Ig) molecules, and each Ig monomer chain has two antigen binding sites so the molecule is bivalent. The hinge region is the area of the heavy chains between the first and second C region domains, and is bonded together by disulfide bonds. The length of hinge region is flexible so the distance between the two antigen binding sites can vary.

The Quartz Crystal Microbalance (QCM)

The quartz crystal microbalance is a simple tool and is increasing in use for mass-sensitive detection due to improvements in experimental procedures [3]. QCMs are used increasingly in liquid phase especially in bioaffinity measurements for analytical or

research purposes. A QCM is a piezoelectric device that can be used to measure specific analytes in contaminated water or in water used to wash contaminated food [1]. The advantages of a QCM are very small size, utility in flow cell, sequential refinement of positive signal, minimal electrical and electronic requirement, adaptability to microfluidic techniques, and inexpensive fabrication [1]. The QCM is a type of acoustic wave sensor. The acoustic wave can propagate in specially cut crystals that mechanically oscillate when subjected to an alternating electric potential. When a proper alternating electrical potential is directed to gold electrodes on opposite sides of the piezoelectric crystal, this crystal will oscillate at a characteristic frequency. The primary theory describing the relationship between frequency change and mass change is described by the Sauerbrey (equation 1). The limit of this equation is the assumption that the mass deposition forms a rigid and thin film and the mass sensitivity is uniform across the full surface.

The QCM can be used as an effective biosensor when the surface of the QCM has been coated with appropriate capture agents, and the capture agents must be stable and tightly associated with the electrode for use in the flow cell. The QCM surface must not react with other substances in the sample. The capture agents must have a high degree of specificity and affinity for the target [1]. Sensitivity and detection limits have to be adequately high to be biologically relevant. For this work, we have used a derivatized self-assembled monolayer (SAM) on the gold surface of the QCM.

Self-Assembled Monolayers (SAMs)

Self-assembled monolayers are continuing to provoke widespread interest in recent years because of their utility in technological applications[6-8]. The areas of current interest include lubrication, adhesion promotion/resistance, corrosion inhibition, lithographic patterning, microelectronic fabrication, wetting adhesion, and for making structures and materials for biological interfaces, membranes and electrochemistry.

Self-assembled monolayers are molecular assemblies that are linked spontaneously to surface phases by the immersion of an appropriate substrate in a solution of an active surfactant in an organic solvent [6]. There are many types of SAM methods including alkanethiols on gold, silver and copper; dialkyl sulfides on gold, dialkyl disulfides on gold; carboxylic acids on aluminum oxide, and silver and silane on quartz and silicon.

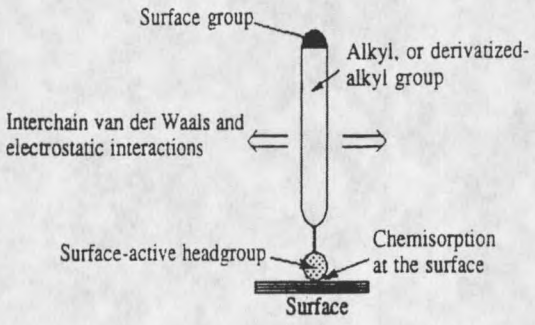

Figure 3. A schematic view of the forces in a self-assembled monolayer

A self assembling surfactant molecule can be separated into three parts in

Figure 3. The first part is the head group that provides the most exothermic process: chemisorption. The strong molecular substrate interactions result in pinning of the head group. This bond can be a covalent bond such as Au-S bond in the case of alkanethiols on gold or ionic $CO_2^-Ag^+$ bond in case of carboxylic acids on AgO/Ag. The energy associated with this bond, for example thiolate on gold, is about 40-45 kcal/mol [6]. The second part is the alkyl chain. Van der Waals interactions are the main forces between simple alkyl chains (C_nH_{2n+1}), on the other hand in some case when a polar group is substituted into the alkyl chain, the electrostatic interactions are energetically more important than the van der Waals force. The last part is the chain terminal group. The structure and orientation of the chain terminating functionality (x) will dictate the surface chemistry of the organic thin film. To provide a simple alkyl chain, the methyl (CH_3) group is widely studied.

Monolayers of Alkane Thiol on Gold

In 1983, Nuzzo and Allara published the first paper in this area. The formation of well-defined organic surface phases by the immersion of a clean gold substrate in a dilute solution of a long chain, ω-substituted dialkyldisulfide showed that dialkyldisulfides (RS-SR) form oriented monolayers on gold surface. Later it was found that sulfur compounds link strongly to gold, silver and copper surfaces as reported in (Table1) [6]. Gold is commonly used because it is reasonably inert which allows substrates to be manipulated in air without concern for contamination. Gold does not have a stable oxide so it can be used in ambient conditions. The reason that SAMs consisting of n-alkane thiol adsorbed on gold are popular is because this system had already proven to be thermally stable,