ELECTROCHEMICAL IMPEDANCE SPECTROSCOPIC ANALYSIS OF BINDING INTERACTIONS BETWEEN HAPTENS AND POLY L-ARGININE AND POLY L-LYSINE

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To my beloved mother and father

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ABSTRACT

Fabrication of an 11-mercaptoundecanoic acid (MUA) self-assembled monolayer (SAM) modified gold electrode in-situ functionalized with poly L-Arginine (pArg) and poly L-Lysine (pLys) is presented and described. The fabricated electrode was used for highly selective and sensitive accumulation and the determination of hapten molecules (Glycerol, Isoeugenol and Maleic anhydride) in a nanomolar concentration. Techniques like cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) with an external redox probe ($FeCN_6^{(3-/4-)}$) were used to investigate the layer-by-layer self assembly modification on a gold electrode, monolayer structure and the ion permeation through it. For CV, Results indicated considerable decrease in current due to immobilizations of amino acid monolayers. In addition, increase in Charge Transfer Resistance (R_{ct}) as main EIS output and Constant Phase Element (CPE) illustrated the quality and the accuracy of Layer-by-Layer (LBL) assembly of SAM monolayers on the surface of gold. EIS study showed the stable readings of R_{ct} relating to pArg and pLys attachment, which was obtained after at least 20 min of immobilization indicating the minimum stability time for amino acid monolayer formation on the gold. In terms of haptenation, increase in R_{ct} showed the direct relation of surface resistance and the degree of hapten sensitization (from weak to strong sensitizers). Eventually, increases in R_{ct} values after attachment of poly l-Arginine SAM monolayer (three vs. two monolayers) illustrated more resistance on the surface of the electrode and more haptenation between probes and haptens. This study can be further used on the development of impedance-based biosensor especially for skin sensitization studies in cosmetic products.

ABSTRAK

Rekaan sebuah *11-mercaptoundecanoic acid* (MUA) *self-assembled monolayer* (SAM) *modified gold* in-situ elektrod yang berfungsi bersama *poly L-Arginine* (pArg) dan *poly L-Lysine* (pLys) telah dibentang dan dihuraikan. Rekaan elekrod ini digunakan untuk pemilihan yang tinggi dan pengumpulan secara sensitif dan penentuan molekul *hapten* (*Glycerol*, *Isoeugenol* dan *Maleic anhydride*) didalam sebuah kepekatan nanomolar. Kaedah seperti *cyclic voltammetry* (CV) dan *electrochemical impedance spectroscopy* (EIS) dengan *external redox probe* ($FeCN_6^{(3-/4-)}$) telah digunakan bagi menyelidik setiap lapisan modifikasi *self-assembly* pada elektrod emas, struktur *monolayer* dan penelapan ion menerusinya. Bagi CV, hasil menunjukkan penurunan arus disebabkan oleh penetapan *monolayer* asid amino. Disamping itu, peningkatan *Charge Transfer Resistance* (R_{ct}) sebagai output utama EIS dan *Constant Phase Element* (CPE) menggambarkan kualiti dan ketepatan penyusunan *monolayer* SAM secara *Layer-by-Layer* (LBL) di atas permukaan emas. Kajian EIS menunjukkan bacaan R_{ct} yang stabil, berkait dengan penambahan pArg dan pLys, yang mana diperoleh selepas sekurang-kurangnya 20 minit penetapan menunjukkan masa kestabilan minimum bagi pembentukan *monolayer* asid amino pada emas. Dalam terma *haptenation*, peningkatan R_{ct} menunjukkan hubungan terus rintangan permukaan dan darjah pemekaan *hapten* (dari pemekaan lemah hingga kuat). Seterusnya, peningkatan pada nilai R_{ct} selepas penambahan *poly l-Arginine* SAM *monolayer* (tiga *vs.* dua *monolayer*) menggambarkan lebih rintangan pada permukaan elekrod dan lebih *haptenation* di antara prob dan *haptens*. Hasil kajian ini boleh diteruskan dalam pembangunan *impedance-based biosensor* terutamanya bagi kajian pemekaan kulit pada produk kosmetik.

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LIST OF SYMBOLS

ɠ	-	Nernst diffusion layer thickness
Б	-	Warburg Coefficient
A	-	surface area of the electrode
AC	-	Alternating Current
ACD	-	Allergic Contact Dermatitis
APG	-	ρ-azidophenyl glyoxal
Bi-PL	-	biotinylatyed-polylysine
CA	-	Cysteamine
C_f	-	Capacity
CMM	-	Capillary membrane model
CPE	-	Constant Phase Element
CV	-	Cyclic Voltammetry
DC	-	Direct Current
DMSO	-	Dimethyl sulfoxide
DNA	-	deoxyribonucleic acid
D_o	-	diffusion coefficient of oxidant
DPV	-	Differential pulse voltammetry
D_r	-	diffusion coefficient of reductant
E.coli	-	Escherichia coli
ECIS	-	Electric Cell-based Impedance Change method
EDC	-	1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide

EDTA	-	Ethylediaminetetraacetic acid
EIS	-	Electrochemical Impedance Spectroscopy
EtOH	-	Ethanol
FRA	-	Frequency Response Analyzer
FT-IRRAS	-	Fourier Transform Infrared Spectroscopy
GA	-	Glutaraldehyde
GGR	-	D-glucose/galactose
Gly	-	Glycine
GPES	-	General Purpose Electrochemical System
HIgG	-	High-G Solution
HMM	-	Homogeneous membrane model
IME	-	Interdigitated microelectrodes
ImT	-	Immersion Time
L.B	-	Lennox broth
LBL	-	Layer-by-Layer
MCT	-	Mercury cadmium telluride
MPA	-	3-mercaptopropionic acid
MPSA	-	3-mercaptopropanesulfonic acid sodium salt
MUA	-	11-mercaptoundecanoic acid
MUD	-	11-mercaptoundecanol
n	-	number of electrons
NHSS	-	N-hydroxysulfosuccinimide ester
NY	-	New York city
pArg	-	poly l-Arginine
PBQ	-	p-benzoquinone
PBS	-	Phosphate Buffer Saline
PCB	-	printed circuit board
PEM	-	polyelectrolyte multilayer modified

pHis	-	poly l-Histidine
pLys/PL	-	poly l-Lysine
PM-FTIR	-	Polarization-modulation Fourier transform infrared spectroscopy
pSS	-	poly sodium 4-styrenesulfonate
pTyr	-	poly l-tyrosine
Ra	-	Resistance
R_{ct}	-	Charge Transfer Resistance
R_p	-	Polarization Resistance
R_s	-	Solution Resistance
SAM	-	self-assembled monolayer
SPR	-	Surface Plasmon Resonance
TEA	-	Thriethanolamine Buffer
UDT	-	1-undecanethiol
UV	-	Ultraviolet
W	-	radial frequency
WI / Z_w	-	Warburg Impedance
XPS	-	X-ray photoelectron spectroscopy
Y_o	-	admittance
Z_d	-	Diffusion impedance

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cosmetic product is one of the most demandable products among people around the world. Products such as moisturizers, shampoos, deodorants, make-up, colognes, and other cosmetics have become part of daily grooming habits. The American Academy of Dermatology reports the average adult uses at least seven different cosmetic products each day. Although cosmetics can help us feel more beautiful, they can cause skin irritation or allergic reactions. Allergic Contact Dermatitis (ACD) is the clinical result of skin contact with chemicals to which an individual is sensitized (e.g. Eczema, burning, stinging or itching without visible skin lesions). This occurs in people who are allergic to a specific ingredient or ingredients in a product. In some cases, the skin becomes red and raw. The face, lips, eyes, ears, and neck are the most common sites for cosmetic allergies, although reactions may appear anywhere on the body. Allergic contact dermatitis occurs because the body's immune system is reacting against a specific substance (haptens) that it considers foreign and harmful. ACD is an important occupational and consumer health problem.

An allergic reaction (happens because of haptenation) is the body's way of responding to an "invader." When the body senses a foreign substance, called

an antigen, the immune system is triggered. The immune system normally protects the body from harmful agents such as bacteria and toxins. Its overreaction to a harmless substance (an allergen) is called a hypersensitivity reaction, or allergic reaction. Certain ingredients used in cosmetics, such as fragrances and preservatives, can act as allergens, substances that trigger an allergic reaction. Reactions may occur in one spot, such as a small-localized skin rash, itchy eyes, face bumps, or all over, as in a whole body rash or hives (urticaria). Allergic reactions are unique for each person. Reaction time to allergens can vary widely. Some people will have an allergic reaction immediately, for others it will take time to develop. Serious cosmetic allergies are rare. However, it is not uncommon for a person to have a mild reaction or irritation to an ingredient in a cosmetic product. Studies suggest that up to 10% of the population will have some type of reaction to a cosmetic over the course of a lifetime. Reactions to cosmetics occur more often in women, most likely because women tend to use more cosmetic products than men do. Thus, checking the products' sensitivity before distribution to the market is required. Some strategies are based on dendritic cells for detecting sensitizing potential of chemicals and some others are based on electrophilic assays. The reaction of nucleophiles with some chemicals inside the product can be useful for detection of the allergens (Xiaobo Yu 2006).

1.2 What is the haptenation?

The term, "hapten," is derived from the Greek "hapten", meaning "to fasten." Haptens are low molecular weight (LMW; <1000 daltons) chemicals that must bind to a carrier molecule to be antigenic. The carrier is usually an endogenous or exogenous protein to which the LMW chemical is covalently bound. The hapten hypothesis was originally proposed to explain both humoral and cellular immune responses to LMW chemicals observed by Landsteiner and Jacobs in their research (Landsteiner and Jacobs 1935). The absolute requirement for covalent binding of a hapten to a protein for immune recognition in the development of all drug/LMW (haptens) chemical allergies has been challenged in recent years, but substantial

evidence exists for this to be a prominent mechanism through which chemicals and drugs or their metabolites become antigenic.

The role of chemical reactivity has been proposed to be one of the major determinants in allergic contact dermatitis (ACD). In the context of occupational health, predictive toxicology, and ensuring overall safety of manufactured products, it is important that skin sensitization potential of new and existing chemicals be assessed. The use of guinea pigs has been the experimental model of choice in evaluating the skin sensitization potential of chemicals until about a decade ago when the local lymph node assay (LLNA) was adopted after extensive interlaboratory validation. Selective protein targets or sites on a protein may be important and recent advances in protein mass spectrometric analysis now provide the capability to better explore how and where such chemicals bind.

1.2.1 How does haptenation occur and change the impedance of the reaction?

The hapten hypothesis was developed from the interaction of nucleophilic moieties on proteins with chemicals that are electrophilic. Approximately 40% of the skin sensitizers have at least an electrophilic center that is amenable to nucleophilic attack. From an organic chemistry perspective, formation of such adducts is via covalent bonds and to a certain extent coordination bonds. This is chiefly because covalent and coordination bonds have bond energies ranging from 200 to 420 kJ/mol compared to hydrophobic, dipolar, and ionic interactions with bond energies <50 kJ/mol. The high bond energies enable covalent adducts to survive the intracellular antigen processing of the haptenated protein into short peptides for cell surface expression by major histocompatibility complexes (MHC). The hypothesis is the haptenation of poly l-Arginine and poly l-Lysine residues with selected haptens due to electrophile-nucleophile interactions. Based on the electrochemistry principles, any interaction between components within solution on electrochemical condition causes the change on the electrochemical equilibrium. Any modification on the

equilibrium changes the impedance property of the reaction and related parameters such as Charge Transfer Resistance (R_{ct}). Thus, it can be one of the useful techniques to detect haptenation within electrochemical condition. In the following research, haptenation within the skin is discussed, as most of the research knowledge gained has been through examining the relationship between chemical reactivity and allergenicity in this organ system.

1.3 Electrochemical Impedance Spectroscopy (EIS) method using Self-assembled monolayers (SAMs)

What is the impedance? "*Electrical impedance is defined as the ratio of an incremental change in voltage to the resulting change in current*." (Jonathan S. Daniels 2007). Electrochemical devices can be modified based on usage and accuracy for the assessments. The main idea is to detect some changes based on electrochemical properties inside the solution (Barreira and Silva 2003, Barreira et al. 2004). Electrochemical based impedance sensing coupled with self-assembled monolayers (SAM) is quite advantageous due to possibility of label free and simple operations. In this research work, poly l-arginine and poly l-lysine self-assembled monolayer (SAM) modified gold electrode was used to detect binding between haptens and poly l-arginine and poly l-lysine through impedance analysis (Barreira et al. 2004). Haptenation of the haptens with poly l-Arginine or poly l-Lysine alters the impedance property and electrochemical equilibrium and leads to create complexes.

Self-assembled monolayers or SAMs is the promising term has been introduced as unstructured way and chemical reaction-based links of integrated molecular building blocks to produce a thickly packed monolayer, stable and highly ordered of molecules from different environments such as gas phase or liquid phase (solution) onto a metal structure (substrate) (Ulman 1996), (Love et al. 2005) and (Denayer et al. 2009). High stability, multi usage (potential to use in various fields)

and simple process of formation on the substrates are the advantages of SAMs method specially used in biosensors construction (Shervedani and Mozaffari 2006), (Mozaffari et al. 2010) and (Chow and Gooding 2006), trace ion determination (Yang et al. 2001), (Berchmans et al. 2000), (Shervedani and Mozaffari 2006), (Shervedani et al. 2009) and (Liu et al. 1999), attachment of biocatalyst (Forzani et al. 2000) and (Shervedani et al. 2006), charge transfer kinetics studies (Protsailo and Fawcett 2000), drug delivery (Crisponi et al. 2010), biomolecules electronic devices (Arya et al. 2009), resistance to corrosion (Laibinis and Whitesides 1992) and also molecular electronics (Kitagawa et al. 2005) and (Chen et al. 1999).

Rapid response, antifouling effects, high sensitivity and even the in-situ attachment of biological recognition elements such as enzymes are major profits of the electrode modification using self-assembled monolayers (SAMs) technique (Mandler and Turyan 1996), (Wink et al. 1997), (Mirsky 2002), (Postlethwaite et al. 1995), (Rahman et al. 2003), (Mohadesi and Taher 2007) and (Wang et al. 2009). Another form of modification was introduced as mixed SAMs on the metal surface. Surface modification by thiols, adsorption of single component and filling the holes (defects) using shorter monolayers was used to overcome the limitations of synthesis and testing of macrocyclic ligands with properties for selectivity of target metal ions (Park et al. 2008).

1.4 Problem Statements

Lysine and Arginine can bind onto the surface of the alkanthiol modified gold electrode because of the strong bonds between the gold and lysine or arginine. However, the binding is just a physical reaction and the instability of the bindings can be assumed. In addition, the revealing of haptens reactivity (haptenation) with the skin proteins such as poly l-Arginine and poly l-Lysine and the possibility of selective detection of the specific type of haptens in terms of sensitivity (weak,

moderate or strong) are the other concerns in this field. This study can lead to the development of a skin sensitizer biosensor.

1.5 Objective

To assess the haptenation between poly l-Arginine and poly l-Lysine Self Assembled Monolayers (SAMs) modified MUA-gold electrode with Glycerol (weak), Isoeugenol (moderate) and Maleic anhydride (strong) skin sensitizers (haptens).

1.6 Scopes of Research

1. Immobilization of 11-mercaptoundecanoic acid (MUA), poly l-Arginine and poly l-Lysine onto gold disk electrode using self-assembled monolayer (SAM) attachment technique.
2. Characterization of poly l-Arginine and poly l-Lysine SAMs modified impedance biosensor using cyclic voltammetry (CV) and electrochemical impedance spectroscopy method.
3. Haptenation analysis of binding interactions between poly l-Arginine and poly l-Lysine and Glycerol (weak), Isoeugenol (moderate) and Maleic anhydride (strong) skin sensitizers using Electrochemical Impedance Spectroscopy (EIS).

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