CONSTRUCTION OF TRIPEPTIDE HETEROLIGAND LIBRARY AS CAPTURING AGENT FOR MERCURY PLASMONIC DETECTION

By

KU SYARIDATUL IRMA BT KU ISMAIL

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair : Amir Syahir Amir Hamzah, PhD
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Mercury is one of the priority metals classified as a human carcinogen by the US Environmental Protection Agency and the International Agency for Research on Cancer. This metallic element has a high degree of toxicity and is known to induce multiple organ damage and have severe adverse effects on human health and the environment, even at low levels of exposure. It has many forms in the soil, including inorganic and organic mercury. In this work, two novel tripeptides were designed and synthesized based on the amino-terminal Cu^{2+} and Ni^{2+} binding (ATCUN) motif. Two systems, namely monoligand and heteroligand systems, were compared in this work. Tripeptides were individually immobilized onto gold nanoparticles (AuNPs) surfaces via covalent coupling. In a monoligand system, only a particular tripeptide-AuNPs will be used as capturing agents for Hg^{2+}, while in a heteroligand system, two different tripeptide-AuNPs will be used simultaneously in a mixture. The heteroligand system was found to be more effective compared to the monoligand system. The interaction of heteroligand enhances the selectivity and sensitivity of the plasmonic sensor for Hg^{2+}. Upon the addition of metal ions, the red-to-blue color change and the degree of AuNPs aggregation formed by the heteroligand system were doubled when compared to the monoligand system. These two novel tripeptides: 0.10 mM of pH 9 DCH (aspartic acid- cysteine-histidine) and 0.20 mM of pH 11 HCD (histidine-cysteine-aspartic acid) were selected among eleven novel tripeptides and one commercial tripeptide as the best capturing agents for Hg^{2+} with an absorbance ratio (A_{683}/A_{524}) of 1.098. The finding was supported by UV-Vis spectra, Dynamic Light Scattering (DLS) spectroscopy, and Transmission Electron Microscopy (TEM) analysis. The limit of detection (LOD) for Hg^{2+} detection was 0.025 parts per millions (ppm) with absorbance reading of 0.094. This new approach can constitute a more effective detection system targeting small molecules such as amino acids, metal ions and fatty acids.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBINAAN PERPUSTAKAAN HETEROLIGAN TRIPEPTIDA SEBAGAI EJEN PENANGKAP UNTUK PENGESANAN PLASMONIK MERKURI

Oleh

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Merkuri adalah salah satu logam utama yang diklasifikasikan sebagai karsinogen manusia menurut Agensi Perlindungan Alam Sekitar A.S. dan Agensi Antarabangsa untuk Penyelidikan Kanser. Unsur logam ini mempunyai tahap ketoksikan yang tinggi, diketahui boleh menyebabkan pelbagai kerosakan organ dan mempunyai kesan buruk terhadap kesihatan manusia dan alam sekitar walaupun pada tahap pendedahan yang rendah. Ia wujud dalam pelbagai bentuk dalam tanah, seperti merkuri tak organik dan merkuri organik. Dalam kajian ini, dua tripeptida novel telah direka dan disintesis berdasarkan motif pengikat terminal amino Cu^{2+} and Ni^{2+} ATCUN. Dua sistem, iaitu sistem monoligan dan heteroligan telah dibandingkan dalam kajian ini. Tripeptida secara individu dialihkan ke permukaan nanopartikel emas (AuNPs) melalui gandingan kovalen. Dalam sistem monoligan hanya satu tripeptida-AuNP tertentu akan digunakan, manakala dalam sistem heteroligan, dua jenis tripeptida-AuNPs akan digunakan dalam campuran. Sistem heteroligan didapati lebih berkesan berbanding sistem monoligan. Interaksi heteroligand meningkatkan selektiviti dan sensitiviti sensor plasmonik untuk $Hg^{2+.}$ Selepas penambahan ion logam, perubahan warna merah-ke-biru, dan tahap pengagregatan AuNPs yang dibentuk oleh sistem heteroligand adalah dua kali ganda jika dibandingkan dengan sistem monoligan. Kedua-dua tripeptida novel: 0.10 mM of pH 9 DCH (aspartat-sisteina-histidina) dan 0.20 mM of pH 11 HCD (histidina-sisteina-aspartat) ini dipilih antara sebelas tripeptida novel dan satu tripeptida komersial sebagai agen penangkap terbaik untuk Hg^{2+} dengan nisbah penyerapan (A_{683}/A_{524}) sebanyak 1.098. Penemuan ini disokong oleh spektrum UV-Vis, spektroskopi Penyebaran Cahaya Dinamik (DLS), dan analisis Transmission Electron Microscopy (TEM). Had pengesanan (LOD) untuk pengesanan merkuri ialah 0.025 bahagian per million (ppm) dengan bacaan penyerapan 0.094. Pendekatan baharu ini berpotensi untuk membentuk sistem pengesanan yang lebih berkesan, terutamanya dalam menyasarkan molekul kecil seperti acid amino, ion metil dan acid lemak.

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As for myself, congratulations!

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by the Graduate Student

I hereby confirm that:

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LIST OF ABREVIATIONS

As^{3+}	Arsenic (III) ions
ATCUN motif	Amino terminal Cu^{2+} and Ni^{2+}-binding motif
Au	Gold
AuNPs	Gold nanoparticles
BME	2-mercaptoethanol/ ß-mercaptoethanol
Cd^{2+}	Cadmium (II) ions
Co^{2+}	Cobalt (II) ions
-COOH	Carboxyl group
Cr^{3+}	Chromium (III) ions
Cu^{2+}	Copper (II) ions
DNA	Deoxyribonucleic acid
Fe^{3+}	Iron (III) ions
fM	Femtomolar
FOM	Figure of merit
h	Hour
Hg^{2+}	Mercury (II) ions
M	Molar
MDL	Minimum detection limit
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
N	Nitrogen
Ni^{2+}	Nickel (II) ions

nm	Nanometre
$-NH_2$	Amino group
Pb^{2+}	Lead (II) ions
ppb	Part per billion
ppm	Part per million
rpm	Revolution per minute
S	Sulfur
-SH	Thiol group
TEM	Transmission electron microscope
Tripeptide-AuNPs	Tripeptide functionalized AuNPs
UV-vis	Ultraviolet-visible
Zn^{2+}	Zinc (II) ions
%	Percent
µL	Microlitre
℃	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Introduction

Heavy metal pollution harms the environment because of its non-biodegradability and ability to accumulate in living organisms (E. Pehlivan et al., 2009). This is an inevitable cost of industrialization pressures that have increased irresponsible human activities, discharging byproducts directly into the rivers or other water reservoirs (Banares & Alvarez, 2015). Mercury is arguably the most hazardous metal pollutant in the environment. It causes severe diseases due to its physiological toxicity and neurotoxicity effects. The Occupational Health and Safety Authorities (OSHA) across the globe only permit 0.1 ppb level of its exposure, while contacting it at ten ppb will have an immediate danger (Buratti et al., 2019: Priyadarshini & Pradhan., 2017). Hg^0 and Hg^{2+} pollution are common contaminants released from both natural sources such as volcano eruptions and anthropogenic emissions from industrial processes such as mining and fossil fuel combustion (Tangahu et al., 2011). Mercury can be emitted into the air and subsequently settle into the water. More noteworthy, the water-soluble mercury tends to be converted to methylmercury, the most toxic form of mercury, through biomethylation by microorganisms. This will eventually result in contamination of the food chain, which leads to poisoning or even malignancy (Priyadarshini & Pradhan., 2017: Fu et al., 2019). The World Health Organization (WHO) reports that among some subsistence fishing populations, between 1.5/1000 and 17/1000 kids displayed cognitive impairment (severe mental retardation) caused on by fish consumption containing mercury. These included the populations of Brazil, Canada, China, Columbia, and Greenland. In 2010, consumption of methylmercury was linked to 7,360 fatal heart attacks and a 0.14-point decline in the IQ of each foetus, according to a map of Hg-related health concerns in China. Chinese anthropogenic factors are responsible for about 61.8% (4532 fatal heart attacks) and 60.8% (0.08 points) of IQ declines. The remaining statistics relate to emissions from both domestic and foreign anthropogenic sources as well as natural processes such as volcanic eruptions, crustal weathering, and oceanic evasions (Chen, Liang & Liu, 2019). Therefore, close monitoring of mercury-befouled water in real-time becomes a crucial task.

1.2 Problem statement

Moreover, determining the pollutant in the environment is vital to discover a targeted region for the remediation process, which is at the frontline of research (Wang et al., 2020). Several advanced and sophisticated instruments were used to identify heavy metal ions in river or seawater samples, such as microwave plasma atomic emission spectroscopy (MP-AES (Ríos, Peňuela & Botero, 2017), atomic absorption spectrometry (AAS) (Bannon & Chilson, 2001), and inductively coupled plasma mass spectrometry (ICP-MS) (Yamakawa, Moriya & Yoshinaga, 2017). These instruments are operative and reliable. However, they come at a very high cost. Because these facilities are laboratory bound and often operated by highly trained personnel, conducting a robust in-situ analysis is less practical. Therefore, developing a new system that is easy to use and selective toward specific metals of concern is significant.

To detect such small targets, a colorimetric and plasmonic approach using gold nanoparticles (AuNPs) is an optimal method thanks to its unique properties of localized plasmon resonance and plasmon coupling effect (Priyadarshini & Pradhan, 2017). A standalone localized plasma of well dispersed AuNPs gives its solution a red-to-orange range of colors depending on the size and shape of the AuNPs. Meanwhile, when two or more particles come into proximity with a distance of less than 4 nm, plasmon coupling will occur, thus causing the red-shift of the absorbance peak spectra, which is associated with a red-to-purple or red-to-blue color change of the AuNPs solution. Governing such aggregation are molecular interaction forces between the AuNPs-surface bound capturing agent and the target (ions, molecules, etc.). Peptides, aptamers, and DNA are the usual capturing agent candidates to capture a specific target selectively. These capturing agents often serve as monoligands, using only one specific surface-bound bioreceptor. However, the aggregation has an entropic obstacle for the aggregates to be fixed in an optimal condition.

In this study, we propose using a heteroligand system to control the AuNP aggregation more efficiently in detecting target molecules. This study constructs a heteroligand tripeptide library, consisting of seventy-eight pairs of capturing agents combined from eleven novel and one commercial tripeptide. The tripeptides were designed based on the ATCUN (amino-terminal Cu^{2+} and Ni^{2+} binding) motif structure, with a thiol group at the center back of the tripeptide to anchor onto the AuNP surface via thiol-gold interaction. Tripeptides were individually conjugated onto the AuNPs surface and the interaction between seventy-eight pairs of capturing agents upon the addition of ten metal ions was studied. With a combination of two different tripeptides, the heteroligand-functionalized AuNPs detection system formed a more stable complex with the targeted Hg^{2+} than the monoligand systems.

1.3 Objectives

The objectives of this study are:

- To construct a tripeptide heteroligand library as capturing agents for mercury plasmonic detection

    1. To design eleven novel tripeptides that mimics ATCUN motif features for metal ions detection
    2. To optimize the tripeptide-AuNPs in order to enhance the sensing signal
    3. To study the effectiveness of tripeptide-AuNPs in targeting Hg^{2+} and compare between the monoligand and heteroligand system

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