UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND BIOPHYSICAL CHARACTERIZATION OF COPPER(II) AND MOLYBDENUM(V) TETRAPEPTIDES

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FS 2015 28

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By

SHARIFA ZAITHUN BEGUM

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science.

November 2015

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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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November 2015

Chairman: Mohd Basyaruddin Abdul Rahman

Faculty: Science

Metallopeptides have a lot of uses; however, they were questioned for their stability and cytotoxicity as well as their applications in catalysis. Five novel tetrapeptides (P1-P5) were synthesized using the Solid Phase Peptide Scheme (SPPS) and analysed with High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) with percentage purities as high as 99.5%. Three of the peptides (P1-HAAD, P2-HAFD & P3-HAVD) had *N*-terminal protected with fluorenylmethyloxycarbonyl chloride (Fmoc) while the other two peptides (P4-AGHD & P5-PGHD) were not protected. All the peptides were positively charged (+1) and the molecular weight calculated from m/z value of MS results coincided with the theoretical molecular weight of the peptides. Metallopeptides derived from the five novel tetrapeptides with copper acetate monohydrate (MP1-MP5) and molybdenum chloride salts (MP6-MP10) in a 1:2 ratio were synthesised, purified and characterised by Ultraviolet-Visible (UV-Vis) spectroscopy, Ultraviolet-Fluorescence (UV-Fluorescence) spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy, Circular Dichroism (CD) spectroscopy and optical rotation polarimetry. It provided the necessary information on the secondary structure, the successful binding of these two transition metals to the specific amino acids hence leading to the putative geometry of metallopeptides and the difference in the chirality of amino acids, peptides and metallopeptides. These metallopeptides were biophysically characterised and tested for their cytotoxicity against breast cancer cells and the cell proliferation of normal skin cells. After screening with two different breast cancer cells, MDA-MB-231 and MCF-7, only two metallopeptides (MP2 & MP3) were deemed to have potential anticancer properties. The antimicrobial activity of P2 and MP8 were greater than ampicillin against *Escherichia Coli (E.Coli)*. The catalytic activities of the synthesised complexes were evaluated. MP1 & MP3 catalysed both the asymmetric aldol reactions with high enantioselectivity of *p*-nitrobenzaldehyde with cyclohexanone (%ee = 87.3 & 80.3, respectively) and of *p*-anisaldehyde with cyclohexanone (%ee= 95.5 & 90.9, respectively).

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SINTESIS DAN PENCIRIAN BIOFIZIKAL KUPRUM(II) DAN MOLIBDENUM(V) TETRAPEPTIDA

Oleh

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Peptida logam mempunyai banyak kegunaan, tetapi kestabilan, ketoksikan dan aplikasinya di dalam bidang pemangkinan telah dipersoalkan. Lima tetrapeptida baharu (P1-P5) telah disintesis mengikut Skim Fasa Pepejal Peptida (SPPS) dan dianalisis menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC) dan Kromatografi Cecair-Jisim Spektrometer (LC-MS) yang mencapai peratus keaslian setinggi 99.5%. *N*-terminal tiga peptida (P1-HAAD, P2-HAFD & P3-HAVD) telah dilindungi oleh karbonil fluorenilmetiloksiklorida (Fmoc) manakala dua peptida yang lain (P4-AGHD & P5-PGHD) tidak dilindungi oleh Fmoc. Semua peptida adalah bercas positif (+1) dan berat molekul dikira daripada nilai m/z yang diperolehi melalui keputusan MS iaitu bersamaan dengan berat molekul teori peptida. Peptida logam yang diperolehi daripada lima tetrapeptida yang disenaraikan dan garam kuprum(II) asetat monohidrat (MP1-MP5) & molibdenum(V) klorida (MP6-MP10) dalam nisbah mol 1:2 telah disintesiskan, ditulenkan dan dicirikan oleh spektroskopi Ultralembayung-tampak (UV-Vis), spektroskopi Ultralembayung-Pendafluor (UV-Fluorescence), Spektroskopi Inframerah Transformasi Fourier (FTIR), Dikroisme bulat (CD) dan putaran optik polarimetri. Pencirian ini memberi maklumat mengenai struktur sekunder, kejayaan pengikatan kedua-dua logam peralihan kepada asid amino tertentu yang membawa kepada jangkaan geometri peptida logam dan perbezaan kekiralan asid amino, peptida dan peptida logam. Kesemua peptida logam ini telah dicirikan secara biofizikal dan diuji ketoksikannya terhadap sel-sel kanser payudara dan pembinaan sel-sel kulit biasa. Selepas pemeriksaan dengan dua sel kanser payudara yang berbeza, MDA-MB-231 dan MCF-7, hanya dua peptida logam (MP2 & MP3) didapati mempunyai sifat-sifat anti-kanser yang berpotensi. Aktiviti antimikrob P2 dan MP8 lebih tinggi daripada ampicillin terhadap *Escherichia coli* (*E.coli)*. MP1 & MP3 memangkinkan kedua-dua tindak balas aldol simetri dengan pemilihan enantio tinggi daripada *p*-nitrobenzaldehid dengan sikloheksanon (%ee masing-masing = 87.3 & 80.3) dan *p*-anisaldehid dengan sikloheksanon (%ee masing-masing = 95.5 & 90.9).

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ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof Dr. Mohd. Basyaruddin, for his utmost guidance, patience, support and for providing me the opportunity to do this research. I would also like to thank my co-supervisors, Dr Mohamed Ibrahim, Dr Bimo and Dr Emilia for their support and contribution in this research. There were a lot to learn through the discussions and excellent advice from my supervisors. Not forgetting WR H[SUHVV P\JUDWLWXGH WR 3URI DWR¶U 6X %DNU Salleh for his encouragement and great advice during our progress meetings.

To all the lab technicians and Chemistry department staff, thank you for everything. I sincerely thank my lab mates from lab 105, my groupmates and my Emtech friends in biotech lab 140 for helping me during my research. To my good friends especially Azren, Zalikha and Rizana, who were there during my ups and downs, who made me smile and laugh, thanking you for all the fun we had, would not be enough. I would also like to thank Miss Tee from Monash University for helping me to run the LCMS of my peptides and Mrs Noraini from IBS (UPM) for helping me to run the cell proliferation test.

Last but not least, I would like to thank my parents who were very understanding at all times. Without their moral support, I would not have completed this project. Thanks would not be enough for all that they have done for me. To my eldest sister; Thahira Begum, words itseOIFDQ¶WH[SUHVVWKHJUDWLWXGH,IHOWGXULQJWKHGXUDWLRQRIWK Thank you for helping me out at the times I needed you the most, despite your busy schedule. Thank you to my other two sisters; Abidha Begum and Husna Begum, for being there for me and continuously giving me encouragement during this period. Without my family, I would not have come up to this level successfully. Thank you all!!

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APPROVAL

I certify that a Thesis Examination Committee has met on 5th November 2015 to conduct the final examination of Sharifa Zaithun Begum on her thesis entitled "SYNTHESIS AND BIOPHYSICAL CHARACTERIZATION OF COPPER(II) AND MOLYBDENUM(V) TETRAPEPTIDES" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

% ee	% enantiomeric excess
⊁@	Optical Rotation
a.a	Amino acid
AAS	Atomic Absorption Spectroscopy
Ala (A)	L-alanine
Arg (R)	L-arginine
Asp (D)	L-aspartic acid
Boc	*Tert*-Butyl-Oxycarbonyl
CD	Circular Dichroism
Cu-Peptide	Copper (II) bound to peptide
Cys (C)	L-cysteine
D-a.a	Dextrorotatory amino acid
d-d	d orbital-d orbital transition
DI	Deionised water
Dr	Diastereomic ratio
FTIR	Fourier Transform Infrared
Glu (E)	L-glutamic acid
Gly (G)	L-glycine
HCTU	O-(6-Chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
His (H)	L-Histidine
HOMO	Highest occupied molecular orbital
HPLC	High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
L-a.a	Levorotatory-amino acid
LC-MS	Liquid chromatography coupled to Mass Spectrometer
Leu (L)	L-leucine
LMCT	Ligand-metal Charge Transfer
Lomets	Local- meta-threading server
LUMO	Lowest unoccupied molecular orbital
Lys (K)	L-lysine
MeOH	Methanol
Met (M)	L-methionine
MLCT	Metal-Ligand Charge Transfer
Mo-Peptide	Molybdenum (V) bound to peptide
MP1	Cu-HAAD-Cu
MP2	Cu-HAFD-Cu
MP3	Cu-HAVD-Cu
MP4	Cu-AGHD-Cu
MP5	Cu-PGHD-Cu
MP6	Mo-HAAD-Mo
MP7	Mo-HAFD-Mo
MP8	Mo-HAVD-Mo
MP9	Mo-AGHD-Mo
MP10	Mo-PGHD-Mo
NMR	Nuclear Magnetic Resonance
P1	HAAD
P2	HAFD
P3	HAVD

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P4	AGHD
P5	PGHD
Phe (F)	L-phenylalanine
Pro (P)	L-proline
Resin	Rink Amide resin (loading capacity: 0.60 mmol/g)
R_T/min	Retention time in minutes
TGA	Thermogravimetric Analysis
Thr (T)	L-threonine
TLC	Thin Layer Chromatography
Trp (W)	L-tryptophan
Tyr (Y)	L-tyrosine
UV-Fluorescence	Ultraviolet Fluorescence
UV-Vis	Ultraviolet Visible Spectroscopy
Val (V)	L-valine

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LIST OF CHEMICAL FORMULAE

ACN	Acetonitrile
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
Conc. HCl	Concentrated Hydrochloric acid
$Cu(CH_3COO)_2.H_2O$	Copper (II) acetate monohydrate
D_2O	Deuterated water
DCM	Dichloromethane
dDMSO	Deuterated dimethyl sulfoxide
DIEA	*N,N-Diisopropylethylamine*
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EDT	Ethane dithiol
EtOH	Ethanol
Fmoc	9-Fluoromethoxycarbonyl
KH_2PO_4	Phosphate buffer
$Ni(NO_3)_2.6H_2O$	Nickel (II) nitrate hexahydrate
$NiCl_2.6H_2O$	Nickel (II) chloride hexahydrate
$NiSO_4.6H_2O$	Nickel (II) sulphate hexahydrate
$NbCl_3$	Niobium (III) chloride
$MoCl_5$	Molybdenum (V) chloride
NMM	N-Methylmorpholine
NMP	N-Methyl-2-pyrrolidone
$RuCl_3.3H_2O$	Ruthenium (III) chloride trihydrate
TFA	Trifluoroacetic acid
TIS	Triisopropyl silane

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CHAPTER 1

INTRODUCTION

Proteins have long been known to have significant benefits to our health. They have been known to possess anti-cancer, anti-microbial, anti-viral and anti-fungal properties. They were also observed to have good catalytic properties in the presence of metal complexes (Lu *et al.*, 2009). However, due to the high amount of energy/time taken and the expensive cost of synthesizing and purifying these metalloproteins, the research in this area did not expand and proliferate until the synthesis and analysis of similar analogues, metallopeptides were reported.

Metallopeptides which are easier and cheaper to synthesise compared to metalloproteins, have caught attention of many researchers. Metallopeptides, synthesized from metal salt and peptides, are known as *pseudo*-proteins. They imitate the applications of large proteins especially in the industrial field of catalysis as well as the biological applications (Ming, 2010).

Peptides have unique chemical and physical properties as they are made from amino acids linked together by amide bonds. To synthesize a peptide, it is important to know the sequence and length of the peptide (which is determined by the number of amino acids) as this can influence the purity and solubility of the peptide. The longer the peptide sequence, the higher the cost of synthesizing and purifying them, hence shorter peptides are generally preferred (Arjmand *et al.*, 2013). Most of the peptides used for the synthesis of metallopeptides were greater than 8 amino acids for better stability. Less steric hindrance was involved as the metal-binding amino acids were spaced apart. Dipeptides bound to metal salts lead to steric hindrance and these complexes could not be applied as catalysts in asymmetric reactions. Hence the peptide should be carefully designed for usage in the synthesis of metallopeptides (Ghadiri, 1993).

There are several ways to design peptide. They can be designed *de novo* or they can be based on the peptide sequences from the native proteins. In this project, the peptides were modified to accommodate a transition metal. The active sites of the peptide were in suitable positions to coordinate with the metal ion. Hence the properties and the conformation of the peptides will change due to this modification (Ball, 2013). It is also important to take into account further applications of these peptides and metallopeptides in catalytic and pharmaceutical industries when designing a peptide.

The studies on the chemistry of metallopeptides have focused largely on retaining three dimensional (3D) structures in a protein once a metal is introduced. A study by Ghadiri (1993) stated that secondary structures of peptides were stabilized with various transition metals. It is better if the metal-binding amino acids (like histidine, cysteine, methionine, etc.) are placed four amino acids apart from each other for a better stability.

There are many applications of synthetic peptides. They are used in polypeptide structure or function studies, antibody production, peptide hormones or hormone analogues, design novel enzymes, drugs and vaccines. Similarly, metallopeptides are used in medicinal, biological and synthetic fields of chemistry. Metallopeptides can be used as antibacterial or antimicrobial agents. Metallopeptides were used for cosmetic purposes such as in tissue remodelling, increasing the protein synthesis of collagen

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(useful for regeneration of youthful skin) and promoting the proliferation of fibroblasts and keratinocytes (Pickart *et al.*, 2012).

In this project, tetrapeptides were synthesized with the aim of placing the metal-binding amino acids in the *N*- and *C*-terminal (i & i+3 positions). Dimetal-peptide adducts were also synthesized. Copper(II) acetate monohydrate and molybdenum(V) chloride were used as the metal salts for the synthesis of metallopeptide. An example (Figure 1.1) shows the predicted structure of a copper metalloprotein (Hureau, 2012), similar to that reported in this work.

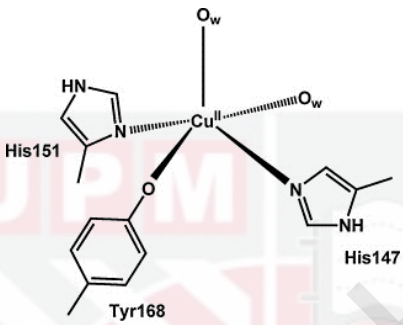

Figure 1.1: Cu(II) coordination in the copper binding domain of Amyloid Precursor Protein (APP) in the transition state [The O_w refers to the oxygen from water, one O_w is a leaving group while a new bond forms with Nitrogen of His151].

1.1 Problem Statements

Three major problems were outlined with reference to stability, toxicity and the catalytic activity of metallopeptides. Short-chain peptides were reported to be unstable compared to longer chain peptides (peptides made up of more than 8 amino acids). This is because longer chain peptides can form a secondary structure (for example alpha-helix or beta sheet) that can stabilize the peptide. Inserting a metal in could bring adverse effects to the metallopeptide by increasing the stability of the short-chain peptides with a random structure by forming extra covalent bonds with the peptide, hence making the structure more rigid. Sometimes the secondary confirmations of the peptide can change from random to . -helix or -sheet structure which is the preferred structure in terms of stability.

In addition, metal bound to short chain peptides can act as good asymmetric catalysts. For example, proline, an amino acid was reported to be a good catalyst with a high enantioselectivity in asymmetric reactions. Another study reported on dipeptides as catalysts in an aldol reaction (Cordova *et al.*, 2005). Dipeptides were modified by adding two more amino acids (containing side chains that could bind to metal) to form tetrapeptides and bind with copper(II) acetate monohydrate and molybdenum(V) chloride (Zou *et al.*, 2005). Since metallopeptides have potential biological applications, in this work, we address their toxicity against cancer cells and also normal skin cells.

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1.2 Objectives

The objectives of this project are:

1. To design, synthesise and characterise tetrapeptides.

2. To synthesise and characterise metallopeptides derived from tetrapeptides and investigate their cytotoxicity against breast cancer cells.

3. To incorporate peptides/metallopeptides as organocatalysts in aldol reactions using different substrates

4. To apply peptides/metallopeptides in other biological applications such as antimicrobial assays and skin cell proliferation studies.

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