



UNIVERSITI PUTRA MALAYSIA

**ISOLATION, CLONING AND EXPRESSION OF RECOMBINANT
HUMAN RENIN IN ESCHERICHIA COLI SYSTEM**

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**ISOLATION, CLONING AND EXPRESSION OF RECOMBINANT HUMAN
RENIN IN *ESCHERICHIA COLI* SYSTEM**

By

NG CHYAN LEONG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Master of Science**

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

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Chairman: Hirzun Mohd. Yusof, Ph.D

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Renin is an important hormone in kidney regulating the renin-angiotensin system (RAS); which plays an important role in human blood pressure. Renin is a highly specific endopeptidase cleaving the Leu-Leu bond in angiotensinogen to generate angiotensin I. Recently, renin was found in organs other than the kidney such as adrenal, ovary, testis, uterus, placenta, anterior pituitary and brain, implicating its involvement in the regulation of numerous activities. Prorenin is the inactive precursor of the renin which regulates the blood pressure and electrolyte balance. Prorenin can be activated *in vitro* following nonproteolysis and proteolysis. The isolation of prorenin or renin from organs including kidney is extremely difficult due to its very low concentration and its instability. Therefore, recombinant protein technologies are used to produce the recombinant human renin protein.

In this study, the full-length human renin coding gene (REN) was isolated from the human kidney cDNA library by using the polymerase chain reaction (PCR) technique. The primers (RF1 & RR1) used were designed based on the human mRNA renin gene sequence from GenBank [gi | 4506474| ref | NM_000537.1]. The



PCR amplified REN gene was cloned into pCR-Blunt cloning vector. Sequencing was carried out and the result shows 99.9% identical to the published sequence. The REN gene was cloned into two different *E. coli* expression vectors, pRSETB and pGEX4T1, to express the recombinant protein. Construct pRB-R was successfully expressed in *E. coli* strains BL21-SI and BL21(DE3)pLysS with the recombinant protein corresponding to the expected size ~48 kDa. Construct pGT-R was expressed in BL21(DE3)pLysS with the size ~66 kDa. Both recombinant proteins have been confirmed with western blotting by using monoclonal anti-His antibody (recombinant protein derived from pRSET vector) and monoclonal anti-GST antibody (recombinant protein derived from pGEX4T1 vector). The result of the expression shows that the combination of the expression vector pRSETB and host BL21(DE3)pLysS gave the highest soluble fraction of recombinant protein.

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

**PEMENCILAN, PENGKLONAM DAN PENGEKSPRESSAN REKOMBINAN
RENIN MANUSIA DALAM SISTEM *ESCHERICHIA COLI***

Oleh

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Renin adalah suatu hormon yang penting dalam ginjal manusia. Ia mengatorkawal sistem renin-angiotensin (RAS) yang mengamalkan peranan penting dalam tekanan darah badan. Renin juga adalah endopeptidase yang sangat spesifik dalam pemotongan ikatan Leu-Leu pada angiotensinogen untuk menghasilkan angiotensin I. Baru-baru ini, renin juga dijumpai di adrenal, ovari, testis, rahim, plasenta, anterior pituitary dan otak. Ini menunjukkan bahawa ia terlibat dalam pengatorkawalan pelbagai aktiviti. Prorenin adalah prekursor renin yang tidak aktif, di mana ia mengawal tekanan darah dan keseimbangan elektrolit. Prorenin boleh diaktifkan *in vitro* mengikut proteolisis dan bukan proteolisis. Pemencilan prorenin atau renin dari organ-organ badan termasuk ginjal adalah sangat sukar disebabkan kepekatan yang rendah dan ketidakstabilannya. Oleh itu, teknologi protein rekombinan telah digunakan untuk menghasilkan rekombinan renin manusia.

Dalam kajian ini, seluruh bahagian pengkodan gen renin manusia (REN) telah dipencilkan daripada perpustakaan cDNA ginjal manusia dewasa (Invitrogen) dengan menggunakan teknik PCR. Pencetus (RF1 & RR1) telah direka berdasarkan

jujukan mRNA gen renin manusia dari GenBank [gi | 4506474| ref | NM_000537.1!]. Hasilan gen renin dari PCR amplifikasi diklonkan ke dalam pCRBlunt vector pengklonan. Penjujukan telah dijalankan dan keputusan menunjuk 99.9% identity dengan jujukan yang telah diterbitkan. Gen REN telah diklonkan ke dalam dua vector pengekspress *E. coli* yang berbeza, iaitu pRSETB dan pGEX4T1, untuk rekombinan protein ekspresi. Binaan pRB-R telah berjaya mengekspress di dalam *E. coli* jenis BL21-SI dan BL21(DE3)pLysS dan menghasilkan rekombinan protein yang saiznya serupa dengan yang dijangkakan, iaitu ~48 kDa. Binaan pGEX4T1 telah mengekpress di dalam BL21(DE3)pLysS dengan saiz ~66 kDa. Kedua-dua rekombinan protein telah disahkan dengan cara western blotting menggunakan monoclonal anti-His antibody (rekombinan protein berasal daripada vektor pRSETB) dan monoclonal anti-GST antibody (rekombinan protein berasal daripada vektor pGEX4T1). Keputusan dari ekpresi menunjukkan bahawa penyantuman vektor pRSETB dan perumah BL21(DE3)pLysS memberi bahagian kelarutan yang tertinggi dalam rekombinan protein.



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I certify that an Examination Committee met on 15th August 2002 to conduct the final examination of Ng Chyan Leong on his Master of Science thesis entitled “Isolation, Cloning and Expression of Recombinant Human Renin in *Escherichia coli* System” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvii

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	4
2.1	Human Kidney	4
2.1.1	Hormone of Human Kidney	4
2.2	Human Renin	5
2.2.1	General Information	5
2.2.2	Basic Molecular Information of Human Renin	6
2.2.3	Prorenin	7
2.2.4	Renin as an Enzyme	7
2.2.5	Renin Angiotensin System (RAS)	8
2.2.6	Expression of Recombinant Human Renin Protein	10
2.3	Production of Recombinant Protein of Various Systems	11
2.4	cDNA library	13
2.5	Polymerase Chain Reaction (PCR)	14
2.5.1	General Information	14
2.5.2	DNA Polymerase used in PCR	15
2.6	<i>Escherichia coli</i>	17
2.6.1	General Information	17
2.6.2	Commercially Available <i>E. coli</i> Expression Vector and Promoter System	17
2.6.3	Heterologous Protein from <i>E. coli</i>	18
2.6.4	<i>E. coli</i> as Expression Host	20
2.6.5	Strain BL21(DE3)pLysS	20
2.6.6	Strain BL21-SI	21
2.6.7	pRSET T7 <i>E. coli</i> Expression Vector	23
2.6.8	pGEX <i>E. coli</i> Expression Vector	24



2.7	T7 Expression System	25
2.8	Ptac Promoter	27
3	MATERIALS AND METHODS	29
3.1	Bacterial Strains and Media	29
3.2	Cloning and Expression Vectors	29
3.3	Isolation of REN Gene from Human Adult Kidney Premade cDNA Library	30
3.3.1	Human Adult Kidney Premade cDNA Library (Invitrogen)	30
3.3.2	Culture of cDNA Library in 96-well plate for PCR template	30
3.4	PCR Primers and Characteristics	32
3.5	Polymerase Chain Reaction (PCR)	32
3.5.1	PCR Screening of the cDNA Library	32
3.5.2	PCR Cloning of REN Gene	33
3.6	Purification of PCR Amplified REN Gene for Cloning and Sequencing	33
3.6.1	Cloning of the PCR- Amplified REN Gene	34
3.7	Analysis and Sequencing of Positive pCRBlunt-R Clones	34
3.8	Plasmid DNA Extraction by Modified Alkaline Lysis Method for <i>E. coli</i>	35
3.9	Agarose Gel Electrophoresis	36
3.10	Quantification of DNA concentration	36
3.11	Restriction Enzyme Digestion	37
3.12	Preparation of <i>E. coli</i> Glycerol Stock	37
3.13	Preparation of <i>E. coli</i> Competent Cell	37
3.13.1	Top 10 and BL21-SI Cells	37
3.13.2	BL21(DE3)pLysS	38
3.14	Sub-cloning of REN Gene	39
3.14.1	PCR Screening of Transformants	39
3.14.2	Sub-cloning of REN gene into intermediate vectors pUC19 and pEG(KT)	40
3.14.2.1	pUC19	40
3.14.2.2	pEG(KT)	40
3.15	Sub-cloning of REN Gene into Expression Vectors (pRSETB and pGEX4T1)	41
3.15.1	pRSETB <i>E. coli</i> Expression Vector	41
3.15.2	pGEX4T <i>E. coli</i> Expression Vector	42
3.16	Summary of Cloning Process	42
3.17	Expression of Recombinant Human Renin Protein in the <i>E. coli</i> Expression Systems	44
3.17.1	Expression of Recombinant REN in <i>E. coli</i> BL21(DE3)pLysS Strain	44



3.17.2	Expression of Recombinant REN in <i>E. coli</i> BL21-SI Strain	44
3.18	Protein Analysis	45
3.18.1	Total Protein Extraction	45
3.18.2	SDS-PAGE Protein Analysis	45
3.19	Western Blotting	46
3.20	Quantification of Protein	47
3.21	Solubility Test of Fusion Protein	48
4	RESULTS AND DISCUSSIONS	49
4.1	Isolation of REN Gene from Human Adult Kidney Premade cDNA Library	49
4.1.1	PCR Based Isolation and Optimisation of REN Gene	49
4.1.2	PCR amplification of REN Gene for Cloning and Express	52
4.1.3	Cloning and Sequencing of PCR Amplified REN Gen	53
4.2	Cloning of Human Renin Gene (REN) into Intermediate Vector and Expression Vector	60
4.2.1	Cloning of Human Renin Gene (REN) into Intermediate Vector pUC19 and pEG(KT)	60
4.2.1.1	pUC19	60
4.2.1.2	pEG(KT)	63
4.2.2	PCR Analysis of REN Gene Clones using Top 10 [pUC19-R] Clones as Template	66
4.2.3	Cloning of REN Gene into <i>E. coli</i> Expression Vector	68
4.2.3.1	Cloning of REN into pRSETB expression vector	68
4.2.3.2	Transformation of pRB-R Recombinant Expression Vector into <i>E. coli</i>	69
4.2.4	Cloning of REN Gene into pGEX4T1 Expression Vector	74
4.3	Expression of Recombinant Human Renin Protein	77
4.3.1	SDS-PAGE and Western Blot Analysis of BL21(DE3)pLysS [pRB-R] and BL21-SI [pRB-R] Clones	77
4.3.2	Recombinant Protein Expression Corresponding to the Time Induction of BL21(DE3)pLysS [pRB-R] and BL21-SI [pRB-R] clones	79

4.3.3	SDS-PAGE and Western Blot Analysis of BL21(DE3)pLysS [pGT-R]	81
4.3.4	Recombinant Protein Expression Corresponding to the Time Induction of BL21(DE3)pLysS [pGT-R] Clone	83
4.4	Solubility Analysis of Recombinant Human Renin Protein	85
4.4.1	BL21(DE3)pLysS [pRB-R] and BL21- SI [pRB-R] Clones	86
4.4.2	BL21(DE3)pLysS [pGT-R] Clone	87
5	CONCLUSION	89
	BIBLIOGRAPHY	91
	APPENDICES	99
	VITA	131



LIST OF TABLES

Table		Page
1	Characteristic of primers.	32

LIST OF FIGURES

Figure		Page
1	Production of angiotensins.	9
2	Direction of pooling 48 wells of human adult kidney premade cDNA library culture used for secondary PCR screening.	31
3	The summary of the cloning process of the REN gene.	43
4	Agarose gel electrophoresis of PCR product using Mastercycler Gradient PCR machine.	50
5	PCR product with using sample pools from 96 well plates culture of <i>E. coli</i> human kidney premade cDNA library as template.	51
6	Agarose gel electrophoresis result of PCR product in wells D1 to D6.	52
7	Agarose gel electrophoresis of PCR product of <i>E. coli</i> human kidney premade cDNA library culture by using <i>pfu</i> DNA polymerase.	53
8	Agarose gel electrophoresis of the PCR product of Top 10 [pCRBlunt-R] clones.	55
9	Agarose gel electrophoresis of the REs analysis result of pCRBlunt-R clone 4.	55
10	The sequencing result of pCRBlunt-R clone from M13 Reverse primer.	57
11	The sequencing result of pCRBlunt-R clone from T7 promoter primer.	58
12	The map of pCRBlunt-R construct.	59
13	PCR and restriction enzymes analysis of Top 10 [pUC19-R] clone.	61
14	The map of the recombinant pUC19-R vector indicating the insertion of the REN gene and the orientation of this gene.	62
15	PCR and REs analysis of recombinant pEG(KT)-R.	64



16	The map of pEG(KT)-R construct indicating the insertion and orientation of the REN gene.	65
17	PCR product by using different dilution factor of broth culture of Top 10 [pUC19-R] clones as template.	67
18	PCR screening result of five Top [10 pRB-R] clones.	69
19	Restriction enzymes digestion analysis of pRB-R construct.	70
20	Restriction enzymes digestion of BL21-SI [pRB-R] clones 1-8 by using <i>Pst</i> I and <i>Hind</i> III.	71
21	PCR screening of clones BL21-SI [pRB-R] using primers RF1 and RR1.	72
22	PCR screening of clones BL21(DE3)pLysS [pRB-R] using primers RF1 and RR1.	72
23	The map of pRB-R, indicating the insertion of REN gene and orientation.	73
24	REs digestions analysis of pGT-R clones.	75
25	PCR result of BL21(DE3)pLysS [pGT-R] clones.	75
26	The map of pGT-R construct, indicating the orientation of the insert.	76
27	SDS-PAGE and Western blot analysis of the over-expressed recombinant protein in BL21-SI [pRB-R] and BL21(DE3)pLysS [pRB-R] clones.	78
28	Expression of recombinant REN in BL21(DE3)pLysS pRB-R corresponding to induction time.	80
29	Expression of recombinant REN in BL21-SI pRB-R corresponding to induction time.	80
30	SDS-PAGE analysis of recombinant REN expression in BL21(DE3)pLysS [pGT-R] clone.	82
31	Western blot analysis of recombinant REN expression in BL21(DE3)pLysS [pGT-R] clone.	82
32	Expression of recombinant REN in BL21(DE3)pLysS [pGT-R] corresponding to induction time.	84



33	Solubility analysis of the recombinant protein expression of pRB-R in BL21-SI and BL21(DE3)pLysS strains after 2 hours induction.	86
34	Solubility analysis of the recombinant protein expression of pGT-R in BL21(DE3)pLysS strain after 2 hours induction.	87



ABBREVIATIONS

A ₂₆₀	absorbance value at 260 nm
ACE	angiotensin converting enzyme
Arg	arginine
Asp	aspartic acid
bp	base pair
BSA	bovine serum albumin
C	cytosine
CaCl ₂	calcium chloride
CBD	cellulose binding protein
<i>ccdB</i>	control cell death gene
cDNA	complementary DNA
CHO	Chinese hamster ovary
DHFR	dihydrofolate reductase
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DsbA	disulfide oxidoreductase
DsbC	disulfide isomerase
EDTA	ethylene diamine tetraacetic acid
EK	enterokinase
EMBL	European molecular biology laboratory
G	guanine
GFP	green fluorescent protein



GST	glutathione-S-transferase
GTE	glucose tris-EDTA
HAT	histidine affinity tag
HCl	hydrochloric acid
Ile	isoleucine
IPTG	isopropylthiogalactoside
kb	kilobase
kDa	kilodalton
LB	Luria-Bertani
Leu	leucine
Lys	lysine
M	molar (mol/L)
mA	milliAmpere
MBP	maltose binding protein
MCS	multi cloning site
MgCl ₂	magnesium chloride
min	minute
ml	milli
mM	millimolar
mRNA	messenger RNA
NaCl	sodium chloride
NCBI	National Center for Biotechnology Information
NEB	New England Biolabs
°C	degree Celsius
OD ₂₆₀	optical density value at 260 nm



OD ₆₀₀	optical density value at 600 nm
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
<i>Pfu</i>	<i>Pyrococcus furiosus</i>
Phe	phenylalanine
POD	peroxidase
Pro	proline
<i>Ptac</i>	<i>tac</i> promoter
PVDF	polyvinylidene fluoride
RAS	renin-angiotensin system
RE	restriction enzyme
REN	full length human renin coding gene
rpm	revolutions per minute
SDS	sodium dodecylsulfate
Ser	serine
SMG	submandibular gland
<i>Taq</i>	<i>Thermus aquaticus</i>
TBS	tris buffered saline
TBST	TBS-tween 20
TEMED	tetramethyl-ethylene diamine
TPA	tissue plasminogen activator
Trx	thioredoxin
<i>Tth</i>	<i>Thermus thermophilus</i>

Tyr	tyrosine
U	units
UPM	Universiti Putra Malaysia
URA	urasil requiring
UV	ultraviolet
v/v	volume per volume
Val	valine
w/v	weight per volume
%	percentage
α	alpha
β	beta
γ	gamma
λ	lambda
ϕ	phi
μg	microgram
μl	microliter
μM	micromolar
x	times



CHAPTER 1

INTRODUCTION

Human renin is an important component of renin-angiotensin system (RAS) in the kidney. It regulates blood pressure and electrolyte balance in the body. Previously, renin was mainly found in kidney and recently it has been reported in several other organs such as adrenal, ovary, testis, uterus, placenta, anterior pituitary and brain; where it is suggested to be involved in the regulation of numerous cellular activities. Renin is also a highly specific endopeptidase enzyme, which only cleaves Leu-Leu bond in angiotensinogen. Due to its unique characteristic as an aspartyl protease and its physiological role in human body and as an important component of RAS system, the understanding of the biochemical and molecular properties of renin is very important. Unfortunately, the low abundance of renin in plasma or kidney extract has limited the analysis and study of renin. Therefore, recombinant DNA technology or genetic engineering plays an important role in producing of this enzyme in abundance to overcome the mentioned problem.

The development of genetic engineering and molecular techniques has resulted in many progresses in the recombinant protein production for pharmaceutical and biochemical compounds. Various expression systems such as bacteria, yeast, virus, plant and animal expression systems have been used to produce recombinant protein. Among these systems, microbial expression systems especially *Escherichia coli* expression system is one of the most widely used due to its many advantages.



Human adult kidney premade cDNA library has been chosen as a source to obtain the full-length human renin coding gene (REN) because kidney is well known as an organ that expresses renin for the RAS system. Therefore the possibility of obtaining the REN gene in this cDNA library is higher.

The polymerase chain reaction (PCR) technique has been chosen to isolate the REN gene. This method provides rapid amplification of DNA fragment for cloning. Besides that, bacterial colonies and plaques with recombinant vector are sufficient as PCR template; hence, isolation can be done directly by using PCR without any DNA preparation (Clackson *et al.*, 1991).

The pCR-Blunt, pUC19 and pEG(KT) cloning vectors were used as intermediate vectors due to their comprehensive multiple cloning sites, which are needed to sub-clone the gene into the expression vector. The vectors with selection markers can be easily identified using appropriate antibiotic.

The host and the promoter that are used in the over-expression of a gene are important elements that affect the yield, stability and solubility of the final product (Balbas & Boliver, 1990). Two *E. coli* strains, BL21(DE3)pLysS and BL21-SI, that use isopropyl- β -D-thiogalactoside (IPTG) and sodium chloride (NaCl) as inducers, respectively, were chosen as expression hosts. The host genetic background, growth requirement (nutrients, temperature and oxygen) and the mode of regulation of gene expression (chemical inducers and nutrient starvation) can play an important role in deciding the final yield of the protein synthesized (Balbas & Boliver, 1990). In this study, pRSETB and pGEX4T1 expression vectors, which use T7 and *tac* as

promoter, respectively, were used to express the recombinant human renin. These vectors contain immuno-detectable oligopeptide sequences or proteins (6xHis-tag and glutathione S-transferase (GST), respectively) as fusion proteins of the REN gene. Therefore, the recombinant proteins can be easily detected using monoclonal antibodies (anti-His and anti-GST antibodies, respectively).

Objectives

The objectives of this study are:

1. To isolate full-length human renin coding gene from human adult kidney cDNA library.
2. To clone the human renin coding gene into the *E. coli* expression vector.
3. To study the expression of the recombinant human renin protein by using the *E. coli* expression system.