UNIVERSITI PUTRA MALAYSIA

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

NG CHYAN LEONG

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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

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

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NG CHYAN LEONG

August 2002

Chairman: Hirzun Mohd. Yusof, Ph.D

Faculty: Food Science and Biotechnology

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 \textit{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

NG CHYAN LEONG

Ogos 2002

Pengerusi: Hirzun Mohd. Yusof, Ph.D.

Fakulti: Sains Makanan dan Bioteknologi


Dalam kajian ini, seluruh bahagian pengkodan gen renin manusia (REN) telah dipenculkan 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].

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Last but not least, my utmost gratitude to my parents and family members for their unfailing love and support whom I am eternally indebted to.
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:

ABDUL MANAF ALI, Ph.D
Professor,  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia.  
(Chairman)

HIRZUN MOHD. YUSOF, Ph.D  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia.  
(Member)

RAHA ABDUL RAHIM, Ph.D  
Associate Professor  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia.  
(Member)

HO CHAI LING, Ph.D  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia.  
(Member)

SHAMSHER MOHAMMAD RAMADILI, Ph.D,  
Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 23 SEP 2002
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follow:

HIRZUN MOHD. YUSOF, Ph.D
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia.
(Member)

RAHA ABDUL RAHIM, Ph.D
Associate Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia.
(Member)

HO CHAI LING, Ph.D
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia.
(Member)

AINI IDERIS, Ph.D,
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
I hereby declare that the thesis is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NG CHYAN LEONG

Date: 28/9/2002
TABLE OF CONTENTS

ABSTRACT ii
ABSTRAK iv
ACKNOWLEDGEMENTS 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 Escherichia coli 17
2.6.1 General Information 17
2.6.2 Commercially Available E. coli Expression Vector and Promoter System 17
2.6.3 Heterologous Protein from E. coli 18
2.6.4 E. coli as Expression Host 20
2.6.5 Strain BL21(DE3)pLysS 20
2.6.6 Strain BL21-SI 21
2.6.7 pRSET T7 E. coli Expression Vector 23
2.6.8 pGEX E. coli Expression Vector 24
3 MATERIALS AND METHODS

3.1 Bacterial Strains and Media

3.2 Cloning and Expression Vectors

3.3 Isolation of REN Gene from Human Adult Kidney Premade cDNA Library
   3.3.1 Human Adult Kidney Premade cDNA Library (Invitrogen)
   3.3.2 Culture of cDNA Library in 96-well plate for PCR template

3.4 PCR Primers and Characteristics

3.5 Polymerase Chain Reaction (PCR)
   3.5.1 PCR Screening of the cDNA Library
   3.5.2 PCR Cloning of REN Gene

3.6 Purification of PCR Amplified REN Gene for Cloning and Sequencing
   3.6.1 Cloning of the PCR-Amplified REN Gene

3.7 Analysis and Sequencing of Positive pCRBlunt-R Clones

3.8 Plasmid DNA Extraction by Modified Alkaline Lysis Method for E. coli

3.9 Agarose Gel Electrophoresis

3.10 Quantification of DNA concentration

3.11 Restriction Enzyme Digestion

3.12 Preparation of E. coli Glycerol Stock

3.13 Preparation of E. coli Competent Cell
   3.13.1 Top 10 and BL21-SI Cells
   3.13.2 BL21(DE3)pLysS

3.14 Sub-cloning of REN Gene
   3.14.1 PCR Screening of Transformants
   3.14.2 Sub-cloning of REN gene into intermediate vectors pUC19 and pEG(KT)
      3.14.2.1 pUC19
      3.14.2.2 pEG(KT)

3.15 Sub-cloning of REN Gene into Expression Vectors (pRSETB and pGEX4T1)
   3.15.1 pRSETB E. coli Expression Vector
   3.15.2 pGEX4T E. coli Expression Vector

3.16 Summary of Cloning Process

3.17 Expression of Recombinant Human Renin Protein in the E. coli Expression Systems
   3.17.1 Expression of Recombinant REN in E. coli BL21(DE3)pLysS Strain
3.17.2 Expression of Recombinant REN in
*E. coli* BL21-SI Strain

3.18 Protein Analysis

3.18.1 Total Protein Extraction

3.18.2 SDS-PAGE Protein Analysis

3.19 Western Blotting

3.20 Quantification of Protein

3.21 Solubility Test of Fusion Protein

4 RESULTS AND DISCUSSIONS

4.1 Isolation of REN Gene from Human
Adult Kidney Premade cDNA Library

4.1.1 PCR Based Isolation and Optimisation
of REN Gene

4.1.2 PCR amplification of REN Gene for
Cloning and Express

4.1.3 Cloning and Sequencing of PCR
Amplified REN Gen

4.2 Cloning of Human Renin Gene (REN) into
Intermediate Vector and Expression Vector

4.2.1 Cloning of Human Renin Gene (REN)
into Intermediate Vector pUC19 and
pEG(KT)

4.2.1.1 pUC19

4.2.1.2 pEG(KT)

4.2.2 PCR Analysis of REN Gene Clones
using Top 10 [pUC19-R] Clones as
Template

4.2.3 Cloning of REN Gene into
*E. coli* Expression Vector

4.2.3.1 Cloning of REN into
pRSETB expression vector

4.2.3.2 Transformation of pRB-R
Recombinant Expression
Vector into *E. coli*

4.2.4 Cloning of REN Gene into pGEX4T1
Expression Vector

4.3 Expression of Recombinant Human Renin
Protein

4.3.1 SDS-PAGE and Western Blot Analysis
of BL21(DE3)pLysS [pRB-R] and
BL21-SI [pRB-R] Clones

4.3.2 Recombinant Protein Expression
Corresponding to the Time Induction
of BL21(DE3)pLysS [pRB-R] and
BL21-SI [pRB-R] clones
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>
<thead>
<tr>
<th>Table</th>
<th>Characteristic of primers.</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Characteristic of primers.</td>
<td>32</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

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

PCR product by using different dilution factor of broth culture of Top 10 [pUC19-R] clones as template.

PCR screening result of five Top [10 pRB-R] clones.

Restriction enzymes digestion analysis of pRB-R construct.

Restriction enzymes digestion of BL21-SI [pRB-R] clones 1-8 by using PstI and HindIII.

PCR screening of clones BL21-SI [pRB-R] using primers RF1 and RR1.

PCR screening of clones BL21(DE3)pLysS [pRB-R] using primers RF1 and RR1.

The map of pRB-R, indicating the insertion of REN gene and orientation.

REs digestions analysis of pGT-R clones.

PCR result of BL21(DE3)pLysS [pRB-R] clones.

The map of pGT-R construct, indicating the orientation of the insert.

SDS-PAGE and Western blot analysis of the over-expressed recombinant protein in BL21-SI [pRB-R] and BL21(DE3)pLysS [pRB-R] clones.

Expression of recombinant REN in BL21(DE3)pLysS pRB-R corresponding to induction time.

Expression of recombinant REN in BL21-SI pRB-R corresponding to induction time.


Western blot analysis of recombinant REN expression in BL21(DE3)pLysS [pGT-R] clone.

Expression of recombinant REN in BL21(DE3)pLysS [pGT-R] corresponding to induction time.
Solubility analysis of the recombinant protein expression of pRB-R in BL21-SI and BL21(DE3)pLysS strains after 2 hours induction.

Solubility analysis of the recombinant protein expression of pGT-R in BL21(DE3)pLysS strain after 2 hours induction.
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
ccdB 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
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
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<tr>
<td>GST</td>
<td>glutathione-S-transferase</td>
</tr>
<tr>
<td>GTE</td>
<td>glucose tris-EDTA</td>
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<tr>
<td>HAT</td>
<td>histidine affinity tag</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>Ile</td>
<td>isoleucine</td>
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<tr>
<td>IPTG</td>
<td>isopropylthiogalactoside</td>
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<tr>
<td>kb</td>
<td>kilobase</td>
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<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani</td>
</tr>
<tr>
<td>Leu</td>
<td>leucine</td>
</tr>
<tr>
<td>Lys</td>
<td>lysine</td>
</tr>
<tr>
<td>M</td>
<td>molar (mol/L)</td>
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<tr>
<td>mA</td>
<td>milliAmpere</td>
</tr>
<tr>
<td>MBP</td>
<td>maltose binding protein</td>
</tr>
<tr>
<td>MCS</td>
<td>multi cloning site</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>magnesium chloride</td>
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<td>min</td>
<td>minute</td>
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<td>ml</td>
<td>milli</td>
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<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
<tr>
<td>NEB</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
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<tr>
<td>OD₂₆₀</td>
<td>optical density value at 260 nm</td>
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</table>
\( \text{OD}_{600} \)  
optical density value at 600 nm

\text{PAGE}  
polyacrylamide gel electrophoresis

\text{PBS}  
phosphate buffered saline

\text{PCR}  
polymerase chain reaction

\text{PEG}  
polyethylene glycol

\text{Pfu}  
\textit{Pyrococcus furiosus}

\text{Phe}  
phenylalanine

\text{POD}  
peroxidase

\text{Pro}  
proline

\text{Ptac}  
\textit{tac} promoter

\text{PVDF}  
polyvinylidene fluoride

\text{RAS}  
renin-angiotensin system

\text{RE}  
restriction enzyme

\text{REN}  
full length human renin coding gene

\text{rpm}  
revolutions per minute

\text{SDS}  
sodium dodecylsulfate

\text{Ser}  
serine

\text{SMG}  
submandibular gland

\text{Taq}  
\textit{Thermus aquaticus}

\text{TBS}  
tris buffered saline

\text{TBST}  
TBS-tween 20

\text{TEMED}  
tetramethyl-ethylene diamine

\text{TPA}  
tissue plasminogen activtor

\text{Trx}  
thioredoxin

\text{Tth}  
\textit{Thermus thermophilus}
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.