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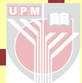
# ISOLATION, CLONING AND EXPRESSION OF RECOMBINANT HUMAN RENIN IN ESCHERICHIA COLI SYSTEM

**NG CHYAN LEONG**

**FSMB 2002 23**



# 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

August 2002

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**

**By**

**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 *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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**Ogos 2002**

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Renin adalah suatu hormon yang penting dalam ginjal manusia. Ia mengaturkawal 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 pengaturkawalan pelbagai aktiviti. Prorenin adalah prekursor renin yang tidak aktif, di mana ia mengawal tekanan darah dan keseimbangan elektrolit. Prorenin boleh diaktifkan *in vitro* mengikuti 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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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

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## 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 biding 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 |

| | |
|---|---|
| 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 biding 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 |
| $^{o}C$ | 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 |
| *Pfu* | *Pyrococcus furiossus* |
| Phe | phenylalanine |
| POD | peroxidase |
| Pro | proline |
| P*tac* | *tac* 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 |
| *Taq* | *Thermus aquaticus* |
| TBS | tris buffered saline |
| TBST | TBS-tween 20 |
| TEMED | tetramethyl-ethylene diamine |
| TPA | tissue plasminogen activtor |
| Trx | thioredoxin |
| *Tth* | *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 |
| $\alpha$ | alpha |
| $\beta$ | beta |
| $\gamma$ | gamma |
| $\lambda$ | lambda |
| $\phi$ | phi |
| $\mu g$ | microgram |
| $\mu l$ | microliter |
| $\mu M$ | 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.