IDENTIFICATION OF SITES OF N-GLYCOSYLATION OF HUMAN AND MOUSE TAMM-HORSFALL GLYCOPROTEIN WITH THE AID OF DATABASE SEARCHING

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THIS DISSERTATION IS PRESENTED TO FULFIL
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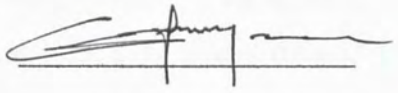

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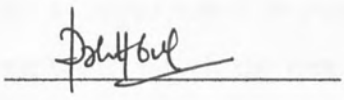

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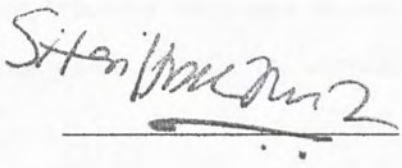

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ABSTRACT

A potential *N*-glycosylation site can be either glycosylated or unglycosylated or partially glycosylated. Therefore, it is important to identify the *N*-glycosylation sites of a glycoprotein in order to understand its function and character. Tamm-Horsfall glycoprotein is the most abundant protein in mammalian urine. The human and mouse Tamm-Horsfall glycoproteins reasonably pure were isolated using Tamm and Horsfall method. The isolation method can isolate Tamm-Horsfall glycoprotein with single band for each sample (HM1, HF1, HF2, and MF1) on SDS-PAGE. All human samples (HM1, HF1 and HF2), have identical size around 100-120 kDa in reduced form. The mouse sample (MF1) has heavier size (around 115-120 kDa) than the human sample. Bradford protein assay was done on a human sample (HM1) and showed that there is 0.0272 milligrams of human Tamm-Horsfall glycoproteins in every millilitre of 0.4 mg/mL of protein solution. The database searching is done to identify the site of *N*-glycosylation. The data of human and mouse Tamm-Horsfall glycoproteins (including precursor) were retrieved from Swiss-Prot database. From the Swiss-Prot database, the expected region of human Tamm-Horsfall glycoprotein (including precursor) to be seen on a human kidney 2D-PAGE was predicted using SWISS-2DPAGE based on the pI and molecular weight computed by the database. The mass of peptide fragments of human and mouse Tamm-Horsfall glycoproteins generated by trypsin with carboxymethylation were computed using PeptideMass. Both human and mouse Tamm-Horsfall glycoproteins *N*-glycosylation sites were identified using NetNGlyc. Human Tamm-Horsfall glycoprotein shows total eight *N*-glycosylation sites (Asn^{38}, Asn^{76}, Asn^{80}, Asn^{232}, Asn^{275}, Asn^{322} Asn^{396}, and Asn^{513}) with all sites predicted to be glycosylated except Asn^{396}. Mouse Tamm-Horsfall glycoprotein shows total ten *N*-glycosylation sites (Asn^{25}, Asn^{38}, Asn^{76}, Asn^{79}, Asn^{233}, Asn^{276}, Asn^{323}, Asn^{397} Asn^{448}, and Asn^{514}) with all ten sites predicted to be glycosylated.

ABSTRAK

Satu lokasi *N*-glikosilasi berpotensi boleh diglikosilasi, tidak diglikosilasi ataupun separa diglikosilasi. Oleh itu, adalah penting untuk mengenalpastikan lokasi *N*-glikosilasi bagi mengetahui fungsi dan sifat suatu glikoprotein. Tamm-Horsfall glikoprotein adalah protein yang paling banyak ditemui dalam air kencing mamalia. Dalam projek ini, Tamm-Horsfall glikoprotein manusia dan tikus telah diasingkan dengan cara Tamm dan Horsfall. Pengasingan adalah agak tulen dengan band tunggal pada SDS-PAGE bagi setiap sample (HM1, HF1, HF2, and MF1). Semua sampel manusia (HM1, HF1 dan HF2), mempunyai saiz yang sama sekitar 100-120 kDa setelah penurunan. Sampel tikus (MF1) mempunyai saiz yang lebih berat (sekitar 115-120 kDa) daripada sample manusia. Bradford protein essei telah dilakukan ke atas sample manusia (HM1) dan menunjukkan bahawa terdapat 0.0272 milligram Tamm-Horsfall glikoprotein manusia dalam setiap milliliter larutan protein 0.4 mg/mL. Data mengenai Tamm-Horsfall glikoprotein manusia dan tikus (termasuk prekusor) telah diperoleh daripada database Swiss-Prot. Lokasi yang dijangka terdapat Tamm-Horsfall glycoprotein manusia pada 2D-PAGE buah pinggan manusia telah diramalkan dengan menggunakan SWISS-2DPAGE berdasarkan pI dan jisim molekul yang dikira oleh database. Jisim serpihan peptide Tamm-Horsfall glikoprotein yang dihasilkan oleh tripsin dan karboksimethilasi telah diperolehi dengan menggunakan PeptideMass. Lokasi *N*-glikosilasi Tamm-Horsfall glikoprotein manusia dan tikus telah dikenalpastikan dengan menggunakan NetNGlyc. Tamm-Horsfall glikoprotein manusia menunjukkan lapan lokasi *N*-glikosilasi (Asn^{38}, Asn^{76}, Asn^{80}, Asn^{232}, Asn^{275}, Asn^{322} Asn^{396}, and Asn^{513}) dengan semua lokasi diglikosilasikan kecuali Asn^{396}. Tamm-Horsfall glikoprotein tikus menunjukkan sepuluh lokasi *N*-glikosilasi (Asn^{25}, Asn^{38}, Asn^{76}, Asn^{79}, Asn^{233}, Asn^{276}, Asn^{323}, Asn^{397} Asn^{448}, and Asn^{514}) dengan semua lokasi diglikosilasikan.

CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF PHOTOS

LIST OF SYMBOLS

%	percent
°C	degree Celsius
g	gram
mg	milligram
min	minute
h	hour
L	litre
mL	millilitre
µL	microlitre
cm	centimetre
mm	millimetre
µm	micrometre
nm	nanometre
Da	Dalton
kDa	kilo Dalton
M	Molarity
rpm	rotation per minute
$mLmin^{-1}$	millilitre per minute
$mgmL^{-1}$	milligram per millilitre
V	volt
v/v	volume per volume

w/w	weight per weight
m/z	mass to charge ratio
ER	endoplasmic reticulum
Man	mannose
GlcNAc	*N*-Acetylglucosamine
GalNAc	*N*-Acetylgalactosamine
Ser, S	serine
Thr, T	threonine
Asn, N	asparagine
Pro, P	proline
Asp, D	aspartic acid
GPI	glycosylphosphatidylinositol
EGF	epidermal growth factor
ZP	zona pellucida
DNA	deoxyribonucleic acid
cDNA	complementary deoxyribonucleic acid
PNGase F	peptide-*N*-glycosidase F
RP-HPLC	reversed-phase high pressure liquid chromatography
MALDI-TOF	matrix assisted laser desorption/ionization-time-of-flight
APS	ammonium persulphate
TEMED	N,N-tetramethylenediamine
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis

CHAPTER 1

INTRODUCTION

Glycoprotein is a type of protein where it has carbohydrate chains attached to it. Most proteins synthesized in eukaryotic cells are glycoproteins. The process of carbohydrate chains attached to protein is called glycosylation. Glycoprotein can be classified as either *N*-linked or *O*-linked (Cooper and Hausman, 2004), depending on the site of attachment of the carbohydrate chains attached to the protein. The carbohydrate chains are attached to glycosylation sites on the protein. However, not all glycosylation sites are glycosylated. The functions of glycoprotein are dependent on the structure of the carbohydrate chains and the sites where the carbohydrate chains are attached to. Although sequencing can yield the sequence of a protein to identify the glycosylation sites, but whether the potential glycosylation sites are actually glycosylated and the functions of it are not known.

Tamm-Horsfall glycoprotein is the most abundant protein found in mammalian urine. The information on Tamm-Horsfall glycoprotein is still in its juvenile stage. There are still more to be explored on this glycoprotein. In previous studies, most of its activities are related to its carbohydrate chains. Different types of carbohydrate

chains have different functions and different carbohydrate chains are attached at different glycosylation sites but not all glycosylation sites are attached by carbohydrate chains. Therefore, glycosylation sites affect the function of a glycoprotein. Since *N*-linked carbohydrate chains are the most common carbohydrate chains found in glycoproteins of most of the animal species, detailed analysis on the *N*-glycosylation sites of Tamm-Horsfall glycoprotein should be done in order to contribute to the unravelling biological functions of Tamm-Horsfall glycoprotein.

Bioinformatics is the term used to refer to the combination of methods in biology, computation, and information management, which are necessary in advance research relating to all aspects of living systems, from individual molecules, cells, and organs to entire organisms. Today, research in molecular biology, biotechnology and pharmacology depends on information technology all the way from experiment to the publication of the results. Comprehensive public databases of DNA and protein sequences, macromolecular structure, gene and protein expression levels, pathway organization and cell signalling, have been established to optimise scientific exploitation of the explosion of data within biology. Therefore, database searching is used to investigate the important *N*-glycosylation sites of Tamm-Horsfall glycoprotein.

The objectives of this project are:

1. To isolate human Tamm-Horsfall glycoprotein from urine of normal human.
2. To isolate mouse Tamm-Horsfall glycoprotein from urine of normal mouse.
3. To identify the sites of *N*-glycosylation of human and mouse Tamm-Horsfall glycoprotein with the aid of database searching.

CHAPTER 2

LITERATURE REVIEW

2.1 Glycoprotein

Glycoprotein is named so as it consists of polypeptide chains and carbohydrate chains. The polypeptide chains of glycoprotein are synthesized under genetic control while the carbohydrate chains are enzymatically generated and covalently linked to the polypeptides (Karp, 2002). The quantities of available processing enzymes are generally not sufficient to ensure the synthesis of uniform product of glycoproteins. This results in microheterogenity, where the glycoproteins have variable carbohydrate compositions (Cavallone *et al.*, 2002). The biosynthesis of glycoprotein is called glycosylation.

2.2 Glycosylation

The glycosylation reactions occur in the lumen of endoplasmic reticulum (ER) and in the lumina of the cis-, medial- and trans-Golgi vesicles (Karp, 2002). Generally, the carbohydrate chains are covalently linked to the protein in the ER and then the structures of the sugar residues are altered by either removal or addition in Golgi

apparatus. There are two types of glycosylation, the *N*-glycosylation and the *O*-glycosylation (Cooper and Hausman, 2004).

Most proteins synthesized in mammalian cells are glycosylated. *N*-glycans have only one core structure (refer to Figure 2.1) while *O*-glycans have different core structures (refer to Figure 2.2). The biologically active oligosaccharides are often found on outer chains attached to these cores. Since this project is about *N*-glycosylation, more details will be discussed on *N*-linked glycosylation.

Figure 2.1: Core structure of *N*-glycan [(Man)3 (GlcNAc)2].

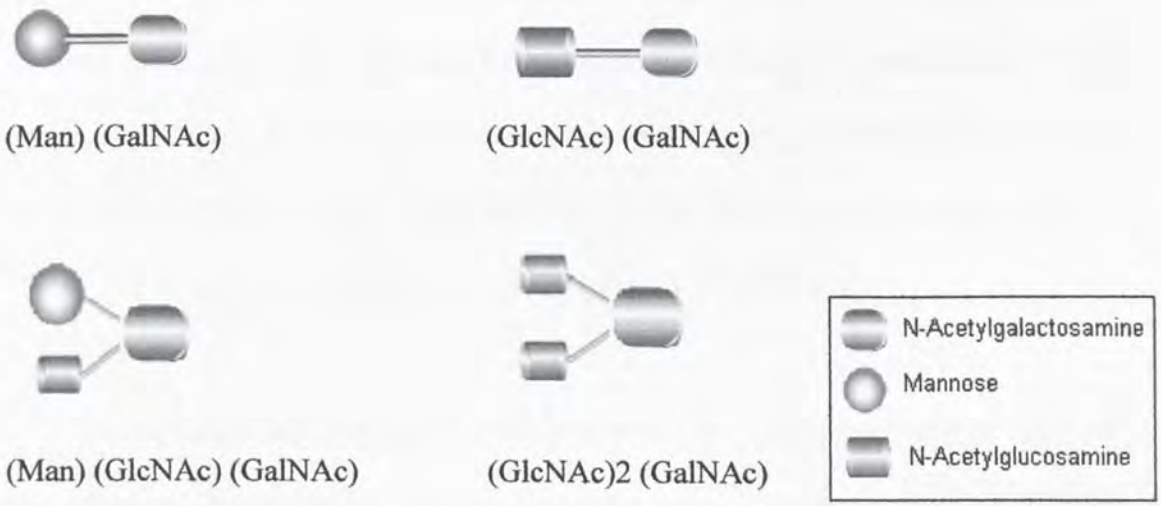

Figure 2.2: Some of the different core structures of *O*-glycan.

2.3 *O*-glycosylation

In *O*-glycosylation, the oligosaccharides that are glycosidically linked to the core protein by covalently bonded to side chain O atoms of specific Serine (Ser, S) or Threonine (Thr, T) residues and are therefore known as *O*-glycosylation. During *O*-glycosylation, carbohydrates are added one at time and each sugar transfer is catalyzed by a different enzyme. It occurs in the Golgi apparatus. The carbohydrate chain by *O*-glycosylation is called *O*-glycan.

2.4 *N*-glycosylation

In *N*-glycosylation, the oligosaccharides are glycosidically linked to the protein via the amide N of specific Asparagine (Asn, N) residues and are therefore known as *N*-glycosylation. The specific Asn residues are either in the sequence X-Asn-X-Ser-X or X-Asn-X-Thr-X, where X is any amino acid except possibly Proline (Pro, P) or Aspartic acid (Asp, D). During *N*-glycosylation, a large oligosaccharide chain containing 14 sugar residues is added to the Asn residue. It occurs in the ER and then, in the Golgi apparatus some sugar residues can be removed and the others can be added. The carbohydrate chain by *N*-glycosylation is called *N*-glycan.

A lipid-linked oligosaccharide precursor is synthesized before the *N*-glycosylation process begins. Its structure is the same in animals, plants, and single cell eukaryotes. The oligosaccharide precursor is linked to dolichol by a pyrophosphoryl group.

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