CHARACTERISATION OF GLYCANS FROM UROMODULIN USING SALT PRECIPITATION METHOD

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DISERTASI INI DIKEMUKAKAN UNTUK MEMENUHI SEBAHAGIAN DARIPADA SYARAT MEMPEROLEHI IJAZAH SARJANA MUDA SAINS DENGAN KEPUJIAN

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JUDUL: CHARACTERISATION OF GLYCANS FROM UROMODULIN USING SALT PRECIPITATION METHOD

IJAZAH: SARJANA MUDA DENGAN KEPUJIAN (BIOTEKNOLOGI)

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DECLARATION

I declare that, except for commonly understood and accepted ideas, or where specific reference has been made to the work of others, this entire dissertation is the result of my own work and includes nothing that is the outcome of collaboration work.

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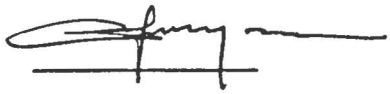

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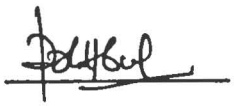

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ABSTRACT

Uromodulin glycoprotein was isolated from urine of pregnant women in different month of pregnancy which are in 9^{th} months and 5^{th} months. The isolation process was done using salt precipitation method originated by Tamm and Horsfall in 1950. When confirming the purity of uromodulin on SDS-page, uromodulin has a molecular weight of 85 to 100 kilo Dalton. The uromodulin isolated from both pregnant women in different months of pregnancy was pure because there are no minor bands formed on the SDS-page gel. Uromodulin glycoprotein was subjected to centrifugation, dialysis and freeze drying to get a dry sample. This project is to characterize the glycans from uromodulin, thus several step was done to examine uromodulin and its glycans. After isolation, steps like reduction and carboxymethylation, tryptic digestion, PNGase F digestion, C_{18} Sep Pak chromatography, reductive elimination and Dowex chromatography was carried out. In addition, experiments like Bradford assay, thin layer chromatography (TLC) separation and serum interaction was also done to examine and observe the characteristic of uromodulin. The result of this experiments showed that the content of uromodulin is high on woman in late pregnancy which is in 9^{th} months and around 9.6 to 10.4 mg/ml after using absorbance of 595 nm in Bradford assay, uromodulin also contain sugar after run on thin layer chromatography plate and uromodulin do not have receptor to interact with serum and therefore not forming agglutination.

ABSTRAK

Uromodulin glikoprotin telah diasingkan dari urin 2 wanita mengandung yang berbeza bulan kandungan iaitu bulan ke-9 dan bulan ke-5. Proses pengasingan urin tersebut telah dilakukan dengan menggunakan kaedah presipitasi garam yang diterbitkan oleh Tamm dan Horsfall pada tahun 1950. Apabila proses untuk memastikan ketulinan uromodulin yang diasingkan pada SDS-Page, didapati uromodulin mempunyai berat molekular diantara 85 hingga 100 kilo Dalton. Uromodulin yang diasingkan dari 2 wanita yang berbeza bulan kandungan adalah tulin kerana tiada terbentuknya belang minor pada gel SDS-Page. Uromodulin glikoprotin telah melalui proses emparan, dialisis dan pengeringan beku untuk mendapatkan sampel yang kering. Projek ini bertujuan untuk meganalisis karekteristik glikan dalam uromodulin, oleh itu beberapa langkah telah dijalankan untuk menguji uromodulin dan glikan. Selepas proses pengasingan, langkah-langkah seperti reduksi dan karboksimetilasi, pemotongan triptik, pemotongan PNGase F, kromatografi C_{18} Sep Pak telah dijalankan. Selain itu, eksperimen seperti asei Bradford, pengasingan TLC dan interaksi dengan serum telah dilaksanakan untuk menguji dan memerhati karekteristik uromodulin. Keputusan eksperimen telah menunjukkan kandungan uromodulin adalah tinggi pada wanita di hujung kandungan iaitu pada bulan ke-9 iaitu diantara 9.6 hingga 10.4 mg/ml dengan menggunakan penyerapan pada 595 nm dalam asei Bradford, uromodulin juga mengandungi gula selepas diasingkan pada plat TLC dan uromodulin tidak mempunyai reseptor untuk interaksi dengan serum, oleh itu tidak membentuk aglutinasi.

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LIST OF SYMBOLS

%	percent
mg	milligram
kDa	kilo Dalton
M	molar
°C	degree Celsius
Rpm	revolution per minute
ml	milliliter
cm	centimeter
nm	nanometer
g	gram
µm	micrometer
L	liter
mM	milimolar
µL	micro liter
V	volt

LIST OF ABBREVIATIONS

THP	Tamm-Horsfall glycoprotein
GPI	Glycophosphatidylinositol
GlcNAc	N-Acetylglucosamine
Asn	Asparagine
Ser	Serine
Thr	Threonine
Pro	Proline
Glc	Glucose
GalNAc	N-Acetylgalactosamine
Man	Mannose
Hyl	Hydrolysine
Hyp	Hydroxyproline
Gal	Galactose
ECM	Extracellular matrix
LFA-3	Lymphocyte function associated antigen-3
ER	Endoplasmic Reticulum
UDP-GlcNAc	Uridine diphosphate-N-acetylglucosamine
ZP	Zona pellucida
TEMED	N'-tetramethylenediamine

CHAPTER 1

INTRODUCTION

1.1 Introduction

The history of glycoprotein started in the year 1895 where Morner discovered and recognized the presence of mucosubstance in normal human urine. A mucoid substance, 'mucoidahnliche Substanz' was isolated from nubecula (insoluble mucus substances) which appears in urine on standing. This material contained reducing substances after hydrolysis (Bourrilon, 1972). Then 50 years later, a high molecular weight of glycoprotein was isolated from human urine by Tamm and Horsfall (1950, 1952) and was found to be a potent inhibitor of haemagglutination by myxoviruses (Bourrilon, 1972).

Later uromodulin was found. Uromodulin is an immunosuppressive 85-kilodalton glycoprotein that can be found in urine of pregnant woman. This glycoprotein was found by Muchmore and Decker in 1985 (Devuyst *et al.*, 2005). Since it was found, a lot of researches and experiments have been done to the unique uromodulin. The uromodulin is O-glycosylated glycoprotein that is same with THP or Tamm-Horsfall glycoprotein. At relatively low concentrations, uromodulin was initially shown to inhibit antigen-induced

T cell proliferation (Easton *et al.*, 2000). Furthermore, the glycans associated with uromodulin have been proposed to be essential for its immunosuppressive and cytokine binding activities (Muchmore *et.al.*, 1987).

There were a lot of research has been done in the glycobiology field especially on glycoprotein from human urine which is Tamm-Horsfall and uromodulin glycoprotein. The research has a lot of benefit in answering how a certain disease related to human can occur such as kidney stones and urinary tract infection. Besides, this field of glycoprotein has a lot of potential and perspectives in helping organ transplantation in human. A lot of research and studies had been done in finding answer to this problem. The studies especially focus on uromodulin glycoprotein because it was found in urine of pregnant woman. One studies said that, this glycoprotein (uromodulin) may be why the body of pregnant woman do not reject the pregnancy or the fetus. Moreover the glycoprotein changes in the structure may cause by steroidal hormone during the pregnancy (Easton *et al.*, 2003)

The main objective of this experiment is to characterize the structure of glycans in uromodulin glycoprotein. Glycoproteins are formed by attachment of carbohydrate covalently to many different proteins. Carbohydrates are much smaller percentage of the weight of glycoproteins than of proteoglycans. Many glycoproteins are components of cell membranes, where they play a variety of roles in processes such as cell adhesion and the binding of sperm to eggs (source: http://www.ncbi.nlm.nih.gov/).

1.2 Objectives

The objectives of this research are:

1. To isolate and purify uromodulin from urine of pregnant women in different month of pregnancy (9^{th} and 5^{th} months) using salt precipitation method.
2. To analyze the glycans from uromodulin using Bradford assay, Thin Layer Chromatography (TLC) and serum interaction experiments.

CHAPTER 2

LITERATURE REVIEW

2.1 Glycoproteins

Many glycoproteins carry covalently attached oligosaccharide or polysaccharide chains. There is an astonishing variety of these modified proteins, which are known as glycoproteins, and they serve many different functions. The capacity of carbohydrate can vary from less than one percent to more than 90 percent for different glycoproteins. They have typically one or more chains of monosaccharide units (1 to 30 units long) which can be linear chain or branched. There are numerous different glycoproteins and they are abundant in living organisms appearing in nearly every biological process. Their functions span the entire spectrum of proteins activities, including those of enzymes, transport proteins, receptors, hormones and structural proteins. Most plasma membrane and secretory protein contain carbohydrate chains.

Glycoprotein carbohydrate chains are highly diverse. They are classified into three groups:

1. N-linked oligosaccharides (N-glycans)
2. O-linked oligosaccharides (O-glycans)
3. Glycophosphatidylinositol (GPI) – membrane anchors

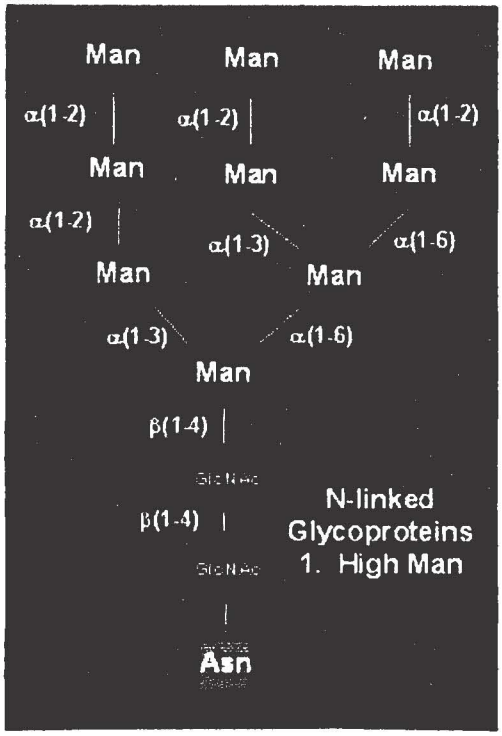
2.2 N-glycans.

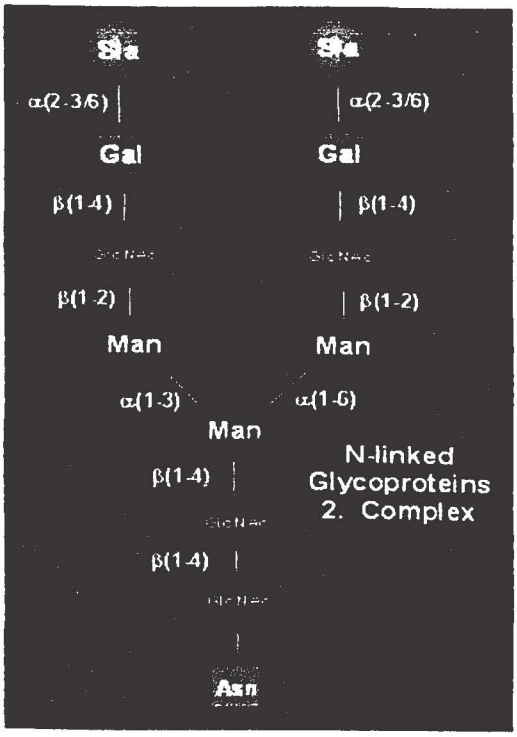
The first sugar residue of N-glycans is usually N-Acetylglucosamine (GlcNAc). It is linked to the amide nitrogen of asparagines (Asn) in the protein. The target sequence for N-glycosylation is Asn – X – Ser/Thr where X can be any amino acid residue except Pro or Asp. This is because Pro side chain would cause the steric hindrance and side chain of Asp is negatively charged; it could make unfavour interactions with negatively charged sugar residues. In some bacterial glycoproteins, Asn residue can be linked to Glc and GalNAc (Resource: Emilia, http://www.cryst.bbk.ac.uk/).

N-glycans are divided into three groups:

1. High-mannose type
2. Complex type
3. Hybrid type

All of them have the common pentasaccharide core and they are synthesized from a common precursor oligosaccharide (Refer to figure 2.1).





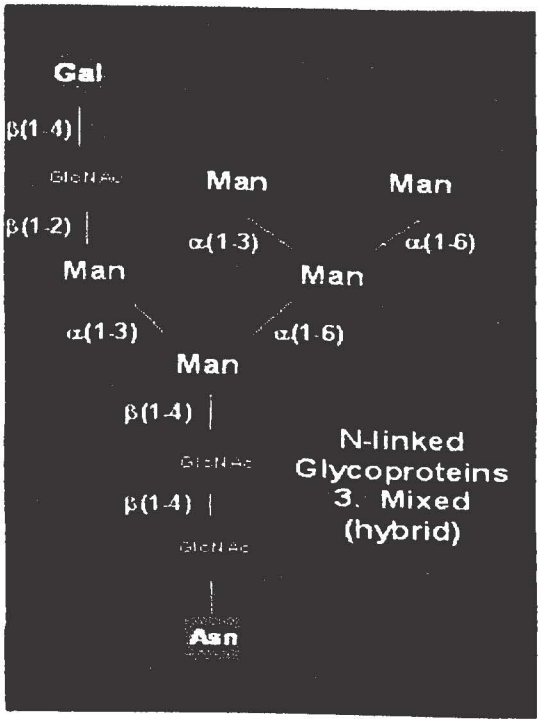


Figure 2.1: Type of N-glycans (Resources: *http://www.users.csbsju.edu/*)

The N-linked oligosaccharides have a minimum of 5 sugar residues. Complex type chains can be mono-, bi-, tri- (2,4 and 2,6 branched), tetra-, and pentaantenary structures in big variety. They can also contain different amount of sialic acid. High-mannose oligosaccharides contain 3 mannose residues to as many as 60 in protozoans and yeast. N-linked glycoproteins exhibit many glycoforms. Cells synthesize many variants of a given glycoprotein. Each variant glycoform differs somewhat in the sequences, locations and numbers of its covalently bound oligosaccharides.

For example an N-linked oligosaccharide chain of RNase B (RNase A is carbohydrate-free) is microheterogenous: a sixth mannose residue occurs at various positions on the core (GlcNAc)2 (Man)5. The carbohydrate does not affect the conformation and substrate specificity or catalytic properties of RNase A.

2.3 O-glyans.

Many proteins carry O-linked oligosaccharides that serve a variety of functions. In O-linked glycosylation, oligosaccharides are attached to a hydroxyl group of:

1. Serine or threonine; then the first sugar residue is usually N-acetylglucosamine (GalNAc). Less commonly, galactose, mannose or xylose form O-glycosidic bonds with Ser or Thr. Although most cytosilic acids and nuclear proteins are not glycosylated, when they are, a single N-Acetylglucosamine residue is linked to the Ser or Thr hydroxyl group. These exceptions include some nuclear-pore complex proteins and some transcription factors.

2. Hydrolysine (Hyl); it is glycosylated by the attachment of single Gal residue or glucosylgalactose disaccharide.
3. Hydroxyproline (Hyp); arabinose residue is linked to it.

Hyl and Hyp residues occur only in collagens.

O-linked oligosaccharides are generally short that is 1-4 sugar residues. But for example O-glycans of ABO blood group antigens are longer. The longest O-linked carbohydrate chains occur in proteoglycans. They contain up to 1000 disaccharide units.

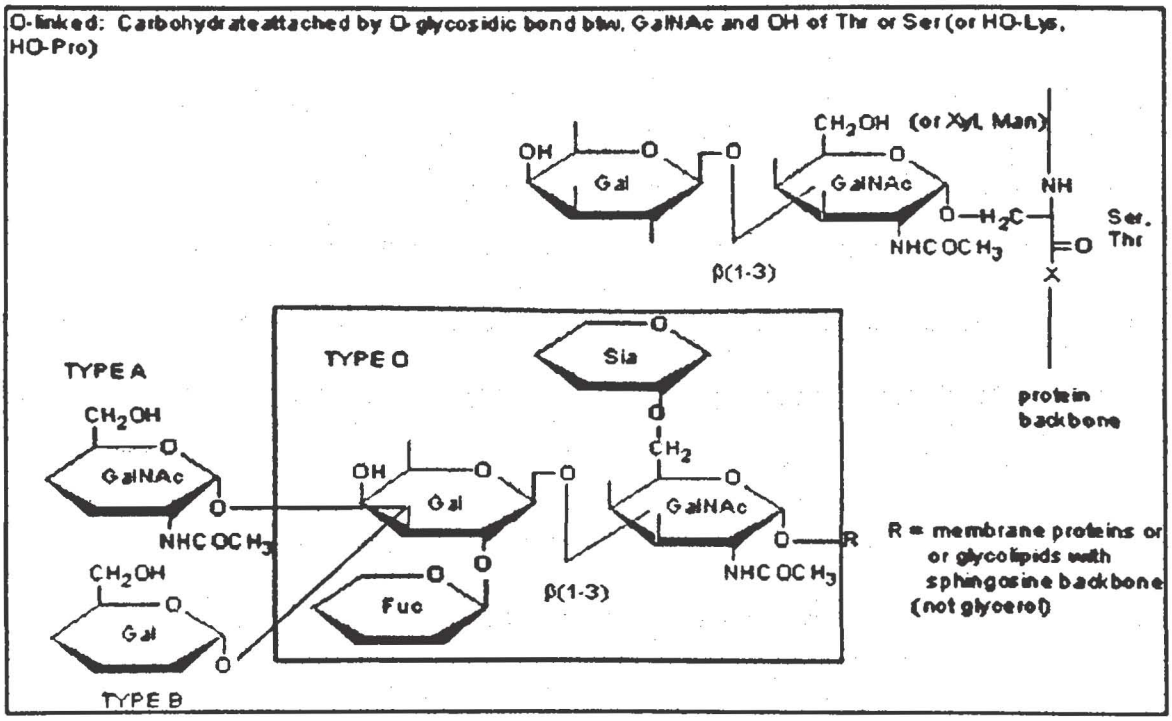


Figure 2.2: Structure of O-glycans (Resource: *http://www.users.csbsju.edu/*)

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