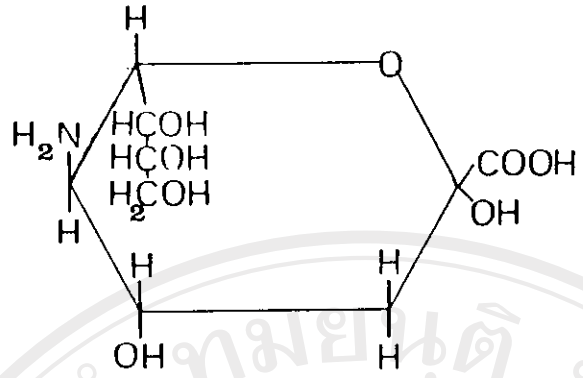
I. INTRODUCTION

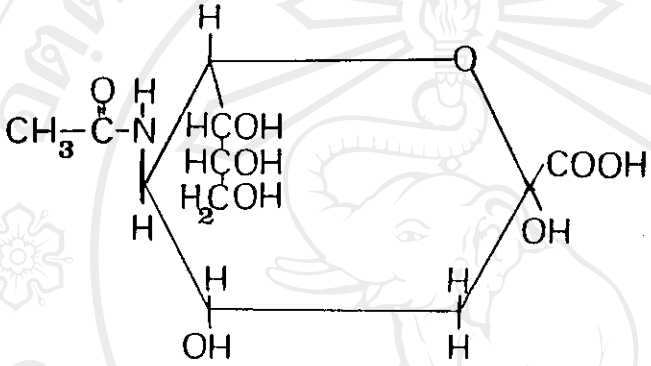
I.1 Sialic Acids

I.1.1 History and Biological Significances of Sialic Acids

Sialic acids are N- and/or O-acyl derivatives of neuraminic acid ($C_6H_{17}NO_8$)(Figure 1a) obtained from natural sources(1). Neuraminic itself is not been found in nature. The name "sialic acid" was created by Blix, Gottschalk and Klenk in 1957 (2). N-acetylneuraminic acid (NANA)(Figure 1b) was first isolated by Gottschalk (3) after the action of influenza virus on ovomucoid or urinary mucin, and by Klenk and coworkers (4) after acid hydrolysis of mucous substances. N-glycolylneuraminic acid (NGNA) (Figure 1c) was isolated from porcine submaxillary gland glycoprotein (5) and several O-acetylated sialic acids were obtained from the corresponding bovine and equine mucins (6). Recently Lapertosa et al. (7) suggested that O-acetylated sialic acids variants are markers of colorectal carcinomas and of an intestinal type of epithelium such as in urinary tract(8).

Sialic acids generally occur in vertebrates and seem do not present in lower animals. They are absent from most bacteria,except E. Coli, Neisseria miningitis and

1a  Neu

1b  NANA

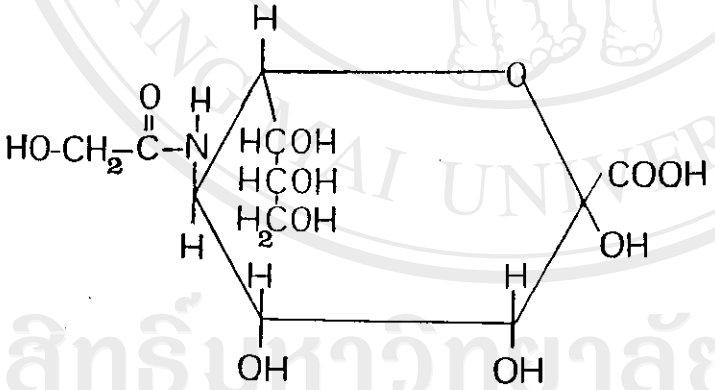
1c  NGNA

Figure 1 Structure of Neu , NANA and NGNA

Samonella strains (9), markedly, all of these are hosted by mammals or are even pathogenic. Sialic acids are also rare in viruses and have never established in plants (10).

Sialic acids naturally occur as glycosidic components of oligosaccharides, polysaccharides and glycoconjugates (10,11). Numerous types of complex carbohydrates in nature are sialoglycoproteins of the O-glycosidic type, and have been found mostly in epithelial cell secretions and in the glycocalyx, and to a lesser extent in serum proteins. Sialic acids are linked to other penultimate β-D-galactose by α(2-3)glycosidic bond or N-acetyl-D-galactosamine by α(2-6)linkage. In contrast, the majority of sialic acids at the cell surface, are linked to penultimate β-galactose by α(2-6) glycosidic bond. Sialic acids are usually bound at terminal position of oligosaccharide chains and structural constituents both of soluble and insoluble components of tissues and cells (10,12). Accordingly, they are formed in glycoconjugates of membraneous structure in cells, including in nucleus. (10)

Free sialic acids usually exist in very low concentrations in biological materials, for example, 50 μM in bovine submandibular gland, 1-3 μM in normal human urine and serum, and 25 μM in human saliva. Much higher concentration of free sialic acids are found in urine, serum and saliva in some human diseases (13, 14) and malignancy (15). Several years ago, a mentally retarded boy excreated

over 10 g of NANA in his urine per day. This value corresponds to an average concentration of sialic acids 45 μM, a value 10,000 times more than that in normal urine. This metabolic disorder is called "Sialuria" (13, 16).

In secreted glycoproteins present in serum, urine and, especially, products from mucous glands also frequently contain a considerable proportion of sialic acids. The content of sialic acids of, for example, human α_1-acid glycoprotein, calf fetuin, edible bird's nest substance or collocalia mucoid and other submandibular gland mucin from several mammals are in the range 9-36% (17).

Interest in Sialic acids has rapidly been increased in recent years, especially since their involvement in the regulation of a great variety of biological phenomena was recognized. Sialic acid residues in glycoconjugates are known or have been proposed to play a key role in normal function of these glycoconjugates. This function includes a role in regulation of protein conformation, ion transport across membrane, protection from proteolytic attack, cell-cell recognition, binding of hormones and other glycolipids to cell adherance of glycoproteins from blood, cellular adhesiveness and binding of virus to cell. Sialic acids also play a role in tumor cell-survival, since their content appears correlated with metastasis ability in a variety of tumor cells.

It is considered that the negative charges of sialic acids may have strong influence on these behavior of cells. This electronegative shield in some cell types, membrane sialic acids prevent aggregation due to electrostatic repulsive in, for example, blood platelets, erythrocytes and carcinoma cells in culture (18). The repulsive, electrostatic forces of sialic acids are also contributed to the rigidity of cell surface, as shown by an increase in the deformability of sarcoma cells after enzymic removal of sialic acid residues (19). Sialic acids seem to facilitate binding of Ca^{2+} compounds to macromolecules and cells, such as, muscle cells (20) and bone tissues. The passage of several substances through the mucous layer on the gut wall was shown to be influenced by the electrical charge of the mucin (21).

Helting et al. (22), Kitamura et al. (23) and Rosner et al. (24,25) founded that sialic acids mainly as a component of gangliosides which was being involved in the binding to cells, of a variety of toxins, such as, tetanus, diptheria, botulinus, (+)-tubocurarine, colchicine and cholera toxin. The B-subunit of cholera toxin binds to the sialoglycoconjugates receptors of the cell membrane,and this is followed by the dissociation of the A-subunit from the native. Toxin molecule then penetrated into that membrane (26).

Similar to the mechanism of above toxin, some glycoprotein hormones were observed that their subunits bind to membrane receptors of the target tissues probably

consisting of sialic acids. These hormone receptors are insulin receptors in liver cells (27, 28), thyroid stimulating hormone and thyroid membrane (26). Besides, sialic acids residues play role as a major determinant in the cellular receptor for influenza viruses (29). Many sialoglycoconjugates are inhibitors of viral hemagglutinin and these inhibitors lost their inhibitory activity after treatment with neuraminidase (sialidase) from influenza viruses or Vibrio cholerae (30). This phenomena was due to a specific interaction between cell surface sialyl residues and viral hemagglutinin. It was considered that sialic acids werecomponent of viral receptors on cell surface.

The anti-recognition effect is one of the most fascinating functions of sialic acids. Ashwell and Morell (31) discovered that sialic acids masking the D-galactose residues of various serum glycoprotein and red blood cell, thus protecting the survival of these molecules in the blood stream. After its enzymic removal, galactose residues were exposed on this molecule and the products are then rapidly recognized by D-galactose specific receptors on the surface of mammalian hepatocytes. This was followed by the fast clearance of the desialylated molecules from circulation (31). The sialic acids side chain seem not to be involved in this function, because shortening by periodate-borohydride treatment does not significantly influence the viability of the red blood cells. It is considered that the carboxyl group plays the main role in the protective effect of sialic acid residues (32).

Further evidence for the masking of antigenic site on cell surface by sialyl residues in reproductive system was observed. On the surface of trophoblast cells, a glycoprotein layer, rich in sialic acids (33) which constitute the boundary zone between maternal and fetal tissues, was considered to form an immunobarrier between the two organism. Thus preventing the formation of antibodies was based on the observation that antibodies against mouse trophoblast cells can be formed in maternal mouse only after enzymic removal of acylneuraminic acid from the surface of these cells.

Another interesting evidence was that accumulation of sialic acids masked the tumor antigens (34). Accordingly, several chemically induced malignant tumor of animals can be brought to regression, or even to disappearance after reinjection of sialidase-treated tumor cells. Similar observation has been made with spontaneous breast tumor of the dog (34). Thus some tumor cells, or their tumor-specific antigens seem to be efficiently masked by sialic acids enabling the transformed cell to escape from immunological attack. Besides, the amount of membrane sialic acids of tumor cells was higher than that of normal cells (35). This finding was fit well with the observations of higher sialyltransferase activities in blood from patients suffering from some kinds of cancer (36). It could be speculated that this oversialylation and probable decrease of cell-surface D-galactose or N-acetylgalactosamine

residues in tumor cells may be one of the reasons for the lack of contact inhibition observed with these cells (36).

It is well known that sialic acids concentration is often increased in plasma or serum of experimental tumor animals or cancer patients (39). Even though the elevations of the concentration are not specific to one type of cancer, but this finding probably reflects the increase in glycoproteins on plasma membrane of malignant cells. It is possible that sialic acids and other carbohydrates are involved in cell contact and recognition phenomena, which are altered in neoplastic cells. The elevation of serum sialic acids were reported in patients with lymphoma, malignant melanoma and cancer of lung as well as prostate, bladder,gastrointestinal system and gynecologic system (40).

Because of their important roles, glycoproteins and glycolipids may be selected to be an appropriate tumor markers. Since cell surface and membrane compounds play a prominant role in neoplastic behavior. Neoplastic cells often have an increased concentration of protein bound sialic acids on their cell surface. Such sialoglyco-conjugates could be shed from the cell into the circulation.

In conclusion, serum sialic acids, as sialoglyco-proteins and sialoglycolipids have been shown to be increased in neoplasm (39). They may be shed from cell membrane surface of cancer cells into blood circulation

relating to tumor staging. Therefore several researchers have paid their attention to the possibility to use serum or tissue sialic acids as a screening tumor marker in various types of cancer diseases.

I.1.2 Determination of Sialic Acids

Various chemical colorimetric methods have been developed for quantitative determination of sialic acids. These techniques are similar in the principle that certain chromogens are formed and react with the reagents in boiling water. Intensity of the colour complex is measured and quantitatively compared to standard curve (using N-acetyl-neuraminic acid or methoxyneuraminic acid as standard). Some methods are suitable for bound sialic acids. These methods are, for example :

1) Direct Ehrlich reaction, using p-dimethyl-benzaldehyde HCl reagent (41).
2) Bial's method, using orcinol and other reactive phenols e.g. resorcinol (42).

Other sugar residues linked to carbohydrate and amino acid in glycoconjugates are found to somewhat interfere in the colour development. Pyrroles, acetamino-furans from 2-amino sugars react with Ehrlich reagent (43), fructose, galactose (44), ketopentose and 2-furfuraldehyde react with resorcinol reagent. Glutaraldehyde, glycoaldehyde hydroxymalonaldehyde also appear to be the substances responsible for interfering colours caused by the periodate oxidation (46).

A more sensitive and the most frequently used method in determination of sialic acids is the periodic acid-thiobarbituric acid (TBA) method devised by Warren (45) and Aminoff (46). This reaction is applicable only to free sialic acids released from sialoglycoconjugates by mild acid hydrolysis or enzyme hydrolysis. Free sialic acids can be assayed in the presence of its bound form. This hydrolysis may lead to some loss of sialic acids ketosidically due to acid hydrolysis or incomplete enzyme release. TBA method is 6-10 times as sensitive as orcinol-Fe^{3+} assay, thus allowing determination of minimal amount of 0.5 g of sialic acids by using micro-adaptation of orcinol assay (47). The nature of chromogen is still unknown but it is more stable and soluble in acidified butane-1-ol (46). Some disadvantage of TBA assay is interference of alkaline arsenite, ethyleneglycol, glycerol, glucose (46), L-fucose (47, 48), unsaturated fatty acid and biliverdin (49). However, N-acetylglucosamine, fructose, aspartic acid, ascorbic acid, serine, threonine, glucosamine-HCl or ribose-5-phosphate were tested and give no interferring colour to TBA reagent (46).

O-acetyl groups of sialic acid side chain (C_7, C_8, and C_9 atoms) also interfere in formation of colour in TBA method by preventing the periodate oxidation (50). For the quantitative determination of total sialic acids their O-acetyl groups must be removed by acid hydrolysis or O-deacetylation in 0.05 M NaOH for 45 min at 0 °C (51). However, O-substituted sialic acids could be

differentially detected by histochemical visualization: the periodic acid-phenylhydrazine-Schiff staining (PAPS). This method is performed by the selective staining of sialic acids in O-acetylated, O-acyl variants and O-sulphate ester forms (52). Besides, sulphated and non-sulphated sialomucin could be distinguished by histochemical staining with high-iron diamine (HID) followed by Alcian blue at pH 1.0 and PAPS respectively (HID-AB 1.0-PAPS)(53). Since sialic acids contain linear glycerol-like side chain which is easily oxidized by periodic acid at a faster rate than cyclic polyols such as in other common monosaccharide. By very mild periodate oxidation the presence of side chain allow highly selective oxidation of sialic acids even when other sugars are present.

Several hereditary metabolic disease known to be deficient or lower activity of neuraminidase are classified as sialisidosis and other disorder of glycoproteins metabolism (54, 55). The diagnosis of these diseases and sialic acid determination of the patient's urine could be measured by the enzymic determination : neuraminidase hydrolysis followed with sialic acid aldolase and NAD^+, NADH specific spectrophotometric method respectively (56). This method is simple enzymic determination of urine sialic acids and specific for free and bound sialic acids without nonspecific colour interference, and the sensitivity of this method can be improved if NAD^+ and NADH are determined fluorometrically.

By the chemical quantitative determination, total forms of sialic acids, usually expressed as NANA, are limited. Each of intact neuraminic acid derivatives e.g. NANA; NGNA; ^{7}O-diacetylNA; N,^{8}O-diacetylNA, and O-sulphated -NA can not be separately detected or assayed by regular chemical method. The biological significance of several various existing forms of sialic acids and their glycosidic structure should not be overlooked and special techniques are therefore needed to quantitatively determine the derivatives.

I.2 Lectins

I.2.1 History and Natural Occurence

Lectins are group of sugar-specific proteins or glycoproteins of nonimmune origin that can agglutinate cells and/or precipitate glycoconjugates. The first lectin was noticed by Stillmark in 1888 (57, 58). While investigating the toxicity of crude extracts of castor bean (Ricinus communis) on blood, Stillmark observed that the red cells were being agglutinated. He named "ricin" to the protein responsible to that agglutination. Afterward, the new lectin of Abrus seeds (Abrus precatorius) was discovered and was named "abrin" (59). These findings raised the interest in studies on plant extracts. Many experiments were shown that some plant extracts could distinguish among human blood groups (60). For example, lima bean lectin would .

agglutinate red cells of only human blood type A, the lotus tetragonolobus lectin specific to type O red cells, and type B specific lectin is from the seeds of Bandeiraca simplicifolia (59,61).

Based on their red cells agglutinating ability, they were called "hemagglutinin" and since they were first isolated from plants, they came to be known as "phytohemagglutinin". Most recently,Boyd and Shapheigh gave the name "Lectin" (Latin : Legere, to select or to pick out) (62,63). Now it has become clear that lectins are present not only in plants, but also in some vertebrates (64, 65, 66, 67) and in invertebrates (68, 69).

In plants, lectins are found primarily in seeds and also present in other part tissues (70). Vertebrate lectin are divided into two classes (71,72) :

a. Integral membrane lectins. None of these has been isolated yet.
b. Soluble lectins.

Invertebrate lectins mainly,are found in hemolymph and sex organs (68, 73). Generally, lectins are usually devided into a small number of specificity groups, according to the monosaccharide that is the most effective inhibitors of the agglutination of red cells or precipitation of glycoconjugates by the lectins, as shown in Table 1. These monosaccharides are D-mannose, D-galactose, N-acetylgalactosamine, L-fucose, N-acetylglucosamine and N-acetylneuraminic

Table 1 Examples of Lectins From Microorganisms, Invertebrates and Plants With Their Specificities

Source	Lectins	Specific saccharides and their conjugates	Reference
1.Microorganisms	Escherichia coli type I	Mannonse polymer	181
	Samonella strains	p-nitrophenyl-α-mannoside	182
	Escherichia coli type P	Gal α 4 Gal	183
	Actinomyces strains	gal, lactose, Galβ3GalNAC methyl-β-galactoside	184
	Mycoplasma strains	Sialoglycoconjugates, oligosaccharides containing sialic acid	185
	Barley Stripe-moseic virus	2-acetamido-2-deoxy-D-hoxose	186
	Streptomyces 2755	unknown	187
	Dictyostelium discoideum	2-acetamido-2-deoxy-D-galactose	188
2.Invertebrated animals	Limulus polyphemus	Sialic acid	189
	Tachypleus gigas	Sialic acid	
	Carcinoscopices rotundicauda	Sialic acid	
	Demania scaberrima	Sialic acid	
	Charybdis natator	Sialic acid	
	Scylla serrata	Sialic acid	
	Parthenope ozakii	Sialic acid	
	Portunus pelagius	Sialic acid	
	Galena bispinosa	Sialic acid	
	Aphonopelma species	Sialic acid	
	Melix pomatia (edible snail)	2-acetamido-2-deoxy-D-galactose	190
3.Plants	Triticum vulgaris	GlcNAc, sialic acid, GalNAc	191
	Pisum sativum	D-mannose, D-glucose	192
	Lens culinaris	D-mannose, D-glucose	193
	Arachis hypogaea	D-galactose	194
	Ricinus communis	D-galactose	195
	Ulex europeus	2-acetamido-2-deoxy-D-glucose	196
	Dolichor bifloius	2-acetamido-2-deoxy-D-glucose	197
	Phaseolus lunatus	2-acetamido-2-deoxy-D-glucose	198
	Ulex europeus II	L-fucose	199
	Canavalia ensiformis	D-mannose, D-glucose	200
	Phaseolus vulgaris	unknown	201
	Agaricus campestris	unknown	202
	Agaricus bisporus	unknown	203

acid. Individual lectins frequently occur as a group of closely related proteins, designated as "isolectins". These isolectins are usually similar in sugar specificity but differ in electrophoretic mobility.

Most lectins contain covalently bound carbohydrates. Two types of glycoprotein lectins have been concerned, these containing primarily mannose and N-acetylglucosamine, and those containing L-arabinose and galactose (74). Many reports suggested that the carbohydrate parts of lectins are not responsible to the lectin activity, based on chemical deglycosylation and enzymatic digestion of carbohydrate chains in several lectins (75,76).

The molecular weight and size of lectins are varying in a wide range. The largest lectin is that from horse-shoe crab (Limulus polyphemus) M.W. : 400,000, and the smallest is 36,000 of the wheat germ lectin. Lectins have no principle structure like immunoglobulins (75). But as a rule, lectins usually consist of subunits, for example, concanavalin A, fava bean lectin and soy bean lectin are composed of 4 subunits, but pea lectin, tomato lectin and wheat germ lectin are composed of 2 subunits (58). The subunits of the same lectin usually have one binding-site for the same specific sugar (73).

Interaction of lectin and sugar is weak and reversible. According to the consisting subunits, lectins can bind to

the glycoconjugates and cell surface carbohydrates than free sugars. The configuration of sugar is important for lectin binding. Many lectins recognize the terminal non-reducing saccharides, while the others also recognize internal sugar sequence (58, 73). A few lectins recognize carbohydrate sequence together with the amino acid to which they are linked, as first demonstrated with the lectin from the mushroom Aparicus bisporus) (77).

The details of subunit structure studies suggest that,some ions are needed to stabilize and protect proteins against aggregation, heat denaturation, enzymatic digestion, conformational change, and agglutinating ability (73). For example,concanavalin A, pea lectin, horse shoe crab lectin and lentil lectin need Ca^{2+} and Mn^{2+}. Mn^{2+} ions stimulated the hemagglutinating activity of lentil (78, 79). Ca^{2+} ions are required for, and sometimes enhanced the agglutination reaction of horse shoe crab lectin (80, 81).

I.2.2 Wheat Germ Lectin

Wheat germ agglutinin (WGA) is a plant lectin from wheat germ (Triticum vulgaris) which is not a blood-group specific lectin. It can agglutinate all types of human erythrocytes as well as a variety of normal and neoplastic cells (82,83). WGA is a basic protein with molecular weight of 36,000, composed of two identical polypeptide subunits (84, 85). Each subunit consists of 164 amino acid residues (M.W.18,000) (86), that are rich in

glycine (24%) and cystein (17%) (87), with 15-17 free SH-groups/mole (88). Some investigating data showed that WGA molecules also contain noncovalently-bond carbohydrate (4.5%). Glucose was found to be a major carbohydrate constituent, but it was not involved in lectin activity (87). . WGA consists of four isolectins which are different in isoelectric point varying from 7.7-8.7(89). The WGA subunit consist of two binding sites, each containing three subsites in a single deep pocket shape. These carbohydrate binding sites are probably situated at the surface of the protein molecule (Figure 2) (84, 85, 90).

The sugars specific to WGA are sialic acid, N-acetyl galactosamine (GalNAc), as well as N-acetylglucosamine (GlcNAc) and its (1-4) linked oligomer. Thus glycoconjugates contain these sugars could agglutinate with WGA (Table 3). It is known that WGA binds to GluNAc with higher affinity compared to NeuNAc and GalNAc respectively 84, 90, 91, 92).

The specific binding of WGA is based on configurational similarities between these sugars. Figure 3 shows the similar structure of GlcNAc, GalNAc and NeuNAc respectively. All are pyranose form, and have the N-acetamido group (C_2 of GlcNAc and of GalNAc; C_5 of NeuNAc) as well as an adjacent hydroxyl groups (C_3, C_4 of GlcNAc and GalNAc and C_4 of NeuNAc). These two groups are in an equatorial position and are in the identical positions of the ring

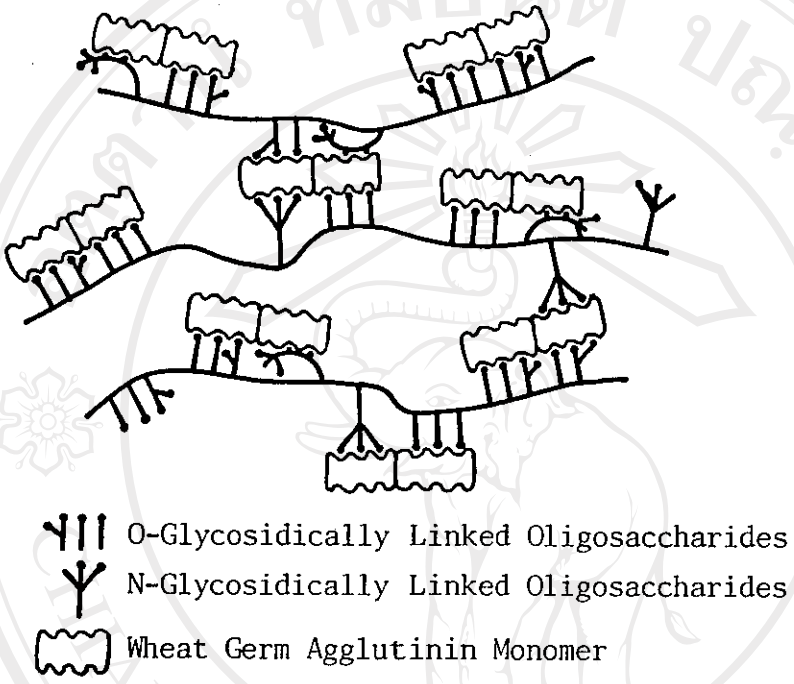


Figure 2 Illustration Representating the Multivalent Interaction of Glycoprotein With WGA. The glycoprotein molecule have oligosaccharide-liked both O-glycosidically and N-glycosidically to the protein.

oxygen, except C_4-hydroxyl group of GalNAc. These groups and their positions are postulated to be responsible in binding of these sugars to WGA (91, 93). This conclusion is also supported with the nuclear magnetic resonance data (94). The C_4-hydroxyl group of GalNAc is in an axial position that partially impairs the binding it to WGA. And the low affinity of NeuNAc in comparison with GlcNAc could come from its hydrophilic tail (C_7-C_8-C_9). After mild periodate oxidizing treatment, the tail shortened to C_7 and the repulsive effect should be weakened. This hypothesis is supported by the oxidized sialoglycoprotein which exhibited high affinity than their untreated counterparts (91,93).

This specific binding has been the basis to several procedures for purifications of WGA by affinity chromatography (95, 96, 97, 98) instead of the ion exchange procedure (CM-cellulose, DEAE-cellulose) (87) or gel filtrations. The GlcNAc-containing molecule have been chosen for this technique. For example: chitin (the naturally occuring polymer of GlcNAc) (97); ovomucoid (a glycoprotein containing GlcNAc from hen egg-white) linked to Sepharose beads (98). From these procedures, WGA was pure enough. The prepared WGA has been used extensively as a probe to study the role of carbohydrate structure and functions of cell membranes.

I.2.3 Horse Shoe Crab Lectin

Limulin (LPA) is a sialic acid binding lectin

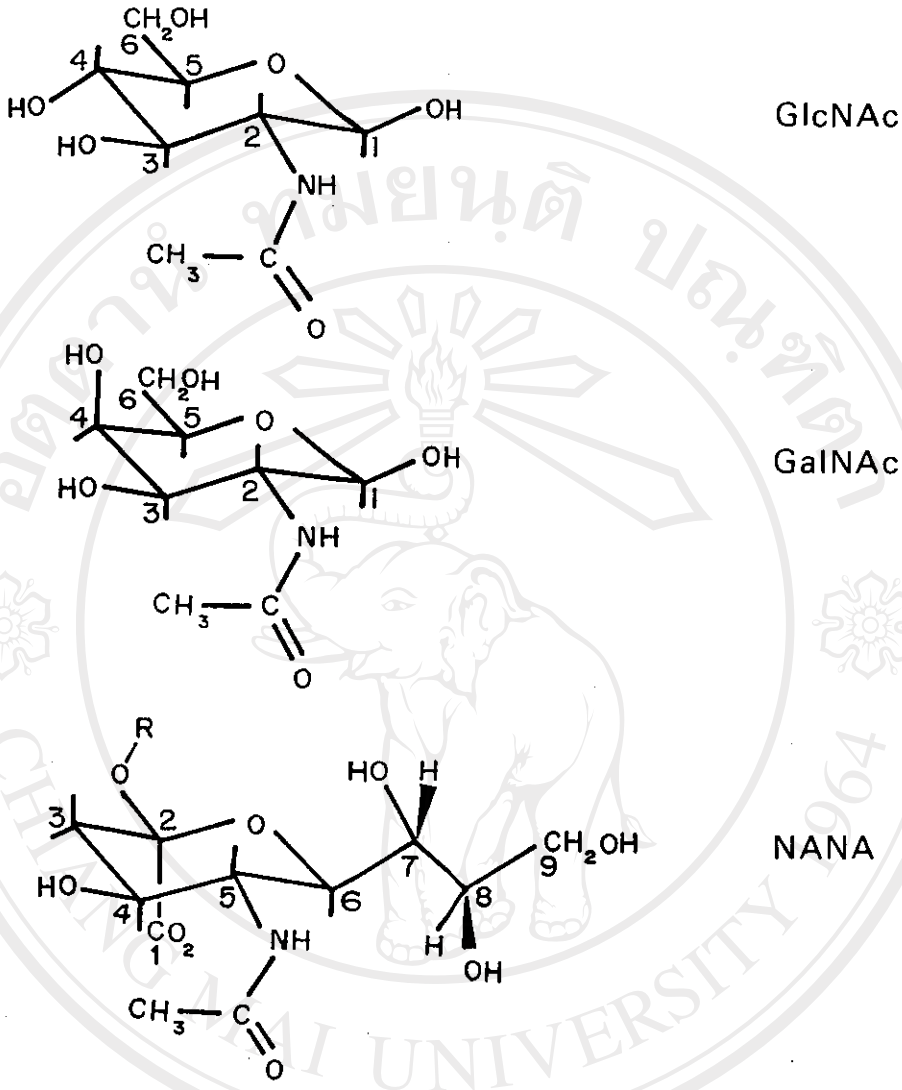


Figure 3 Structure of GlcNAc, GalNAc and NANA

Table 2a Inhibitory Effect of Simple and Complex Saccharides on the Agglutinating Activity of WGA (90).

Compounds	Concentration needed for 50% inhibition (mM)
GlcNAC	12.5
GlcNAc 1-- 4GlcNAc	0.15
GlcNAc 1-- 4GlcNAc-- 4GlcNAc	0.03
ManNAc	200
GalNAc	200
NeuNAc	25
NeuNGc	>200
Glucuronic acid	>332

Table 2b Inhibitory Effect of Glycoconjugates on From Human Serum and Egg White Protein on the Hemagglutinating Activity of WGA(90).

Compounds	Concentration needed for 50% inhibition (mM)
α_1-acid glycoprotein	1.4 ; 4.5
Asialo α_1-acid glycoprotein	>20.8
Fetuin	20.8
Asialo fetuin	>52.7
Ovomucoid	2.2
Ovalbumin	4.2

Table 2c Inhibitory Effect of Glycoconjugates on the Agglutinating Activity of WGA (90).

Compounds	Concentration needed for 50% inhibition (mM)
Glycophorin (MM)*	0.16
Glycophorin (NN)*	0.32
Asialoglycophorin	16.3, > 32.5
Ovine Submaxillary mucin	0.23
Asialo ovine submaxillary	1.85 ; 0.23
Bovine submaxillary mucin	0.32-0.65
Asialo bovine submaxillary mucin	0.32 ; 0.92
Porcine submaxillary mucin	>0.52
Colominic acid	>800
Di-and trisialogangliosides	>1000

* MM and NN designate human blood goonp type O(MM) and O(NN)

from the hemolymph of one of horse shoe crabs (Limulus polyphemus), composed of 5% of total hemolymph protein. It is a glycoprotein lectin containing 4% carbohydrate (2-acetamido-2-deoxyglucose and neutral sugars) (99, 100). The amino acid composition of LPA is rich in glutamic acid, aspartic acid, leucine, and glycine (99, 102). Thus LPA is an acidic protein (pI=4.83) (101). The LPA molecule is represented as ring-shaped structure (Figure 4), in according with electron microscopic data (103). The intact molecule (MW 400,000) can be completely dissociated into 18-20 small subunits (MW 22,500). The three small subunits could become larger subunit (MW 67,000) by covalently binding to each other. Six larger subunits can then be noncovalently bound to form intact molecule. Ca^{2+} ions are required at least 0.001M. The lectin-bound calcium may stabilize the native structure of the protein. Several data suggest that monosaccharide was not a good inhibitor of LPA (99, 100), compared to the sialic acid-containing compounds (101). The following sugars, all tested at 100 mM did not affect the agglutinating ability of purified LPA : N-acetylglucosamine, N-acetylgalactosamine, D-mannose and L-fucose. Free sialic acids could inhibit LPA activity but not as effective as human orosomucoid (containing 10% sialic acids). Sialic acids, 1.5 mM, 10 mM and 40 mM could inhibit agglutination of LPA by 50%, 88% and 100% respectively (101). Human orosomucoid is very potent inhibitor, its concentration as low as 0.005 mM inhibits one unit of agglutination. LPA could also agglutinate erythrocytes,

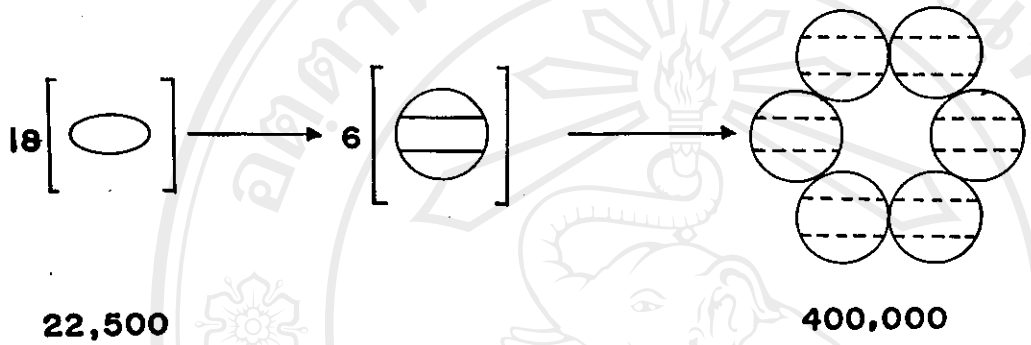


Figure 4 Provisional Structure Model of the Hemagglutinin From Limulus Polyphemus. The molecule is drawn as a ring-shaped structure in accord with the electron micrograph.

human leucocytes, platelets, carcinoembryonic antigen (CEA), and tumor cells (104, 105). D-glucuronic acid is also proved as effective inhibitor. D-glucuronic acid, 3 mM and 40 mM could inhibit LPA agglutination by 50% and 94% respectively (101). LPA binds to both N-acetyl and N-glycolylneuraminic acid (100). It has also been known to be mitogenic toward human peripheral lymphocytes (106).

I.2.4 Biological Roles of Lectins

Although many lectins have been studied and isolated, many questions about lectins are still remained, especially the lectin functions. The possible answers usually come as indirect evidences or as possible postulations.

In plants, lectin functions are proposed as mediators of symbiosis between plants and microorganisms. Many data suggest that lectins are involved in the initiation step of nitrogen-fixation (107, 108). At present, it is found that lectin named, "trifoliin", from clover root (109) could bind to the receptors on root hair cell walls of rhizobium. Another possible function of plant lectin is the involvement in the protection of plants against phytopathogens. This proposal is supported by the evidence that the lectins are present at the invasion site of pathogens. Another supporting is the binding of lectins to various fungi could inhibit fungal growth and germination. For example, wheat germ lectin inhibit the

growth of fungi containing their cell wall chitin, which is N-acetylglucosamine polymer (59). And the germination of Fusarium solani and Trichoderma varide was inhibited (110, 111) when their hyphal tips bind to wheat germ lectin (110).

As physiological roles, plant lectins are thought to be involved in sugar storage, sugar transport and immobilization (60). Some studies showed the appearence and disappearence of lectins during cell cycle of plants (112). Jones and coworkers reported that Maclura ponifera lectin began to accumulate during the early development of seeds, reaches a maximum as the seed achieved maturity, and decreased slowly during germination (112). Castor bean agglutinin is present only in the matrix of protein bodies of the seed embryo (113,114). Lentil lectin occurs during early development and differentiation of seed embryo (115). These evidence raised the proposal that lectins involve in the germination and maturation of embryo.

In animals, membrane lectins are thought to mediate the binding of soluble extracellular and intracellular glycoproteins of cells. In mammalian liver cells, the asialoglycoproteins bind to the galactose-specific lectin (receptor on liver cell surfaces), and asialo-agalactoglycoproteins bind to mannose/N-acetylglucosamine-specific lectin (116). The mannose-6-phosphate-specific lectin mediates the targeting of hydrolytic enzymes to the lysosomes (117). Galactose-specific lectin present on

various human and murine tumors (118-120) were reported to influence the pathogenesis of cancer metastasis by promoting the function of tumor cells aggregates in the circulation and their adherance to the endothelial layer of the cappillaries. The soluble lectins in animals are proposed to bind to the glycoconjugates on and around the cell that release them (72), based on the finding that chicken lactose lectin-I, which is concentrated intracellularly in developing muscles, become extracellular with maturation (121). Another evidence is the finding of rat ß-galactoside binding lectin in lung is concentrated in elastic-fibers, a specific form of extracellular matrix (122).

The appearance of invertebrate lectins in hemolymph or serum, and their ability to recognize the foreign cells, have led to the idea that such proteins may play role in defense mechanism (123, 124). And in some cases, they enhance the phagocytosis (125, 126). Such invertebrates are snails, mollusks , scorpions,spiders, and horse-shoe crabs (127).

The lectins found in bacterial surfaces are also thought to have special function. They act as the mediator for bacterial adherance to epithelial cells, for example, in urinary and gastrointestinal tract. The pattern of ditribution on oral epithelial surface of actinomyces carrying the galactose (lactose)-specific lectin supports this assumption that these lectins are the principal

mediators of adherance, colonization and establishment of specific microbial communities in oral cavities by binding to streptococci and to epithelial cells (128).

I.2.5 Applications of Lectins

The early applications of lectins, still in wide use, were blood typing (60),separating leucocytes from erythrocytes (129), cell fractionation and agglutination of cells from blood in preparation of plasma (54). According to its mitogenic activity toward lymphocytes, lectins were used as tools for the study of biochemical and immunological events accompanying cell growth, cell differentiation and division (131, 132).

The ready availability of a large number of lectins with different sugar specificities has led to their extensive utilization as reagent for the study of simple and complex carbohydrates, in both soluble and on cell surface for identification, isolation, characterization,distribution and mobility. Lectins are good tools the study of insulin receptor on rat adipocytes with contained sialic acids by wheat germ lectin (132), lectin from horse shoe crab (133) and lima bean lectin including lectin (134). Some lectins are used for study the modification of chemotaxis of nematodes as mediators (135). In isolation of glycoproteins, immobilized lectins were used in affinity chromatography.

for example, insulin (136), epidermal growth factor (137), glycocalicin (the predominant glycoprotein of human platelet membrane (140).

The usefulness of lectins can be greatly increased by using conjugated lectins or labelled lectins. Because lectins exhibit very discrete carbohydrate binding and the carbohydrate are relatively resistant to histologic fixation, labelled-lectins are powerful tools to define the carbohydrate on tissue membranes. An individual lectin is conjugated to visualants, such as ferritin, isotopes, fluorescein dyes, biotin, enzymes (e.g. alkaline phosphatase and horse radish peroxidase), gold particles or any electron-dense substances (138, 139). There are numerous reports on the application of lectin in histochemical and cytochemical studies. Changes in lectin binding pattern have been observed during cell maturation (140), aging (141), neoplastic alternation (142), malignant transformation (89, 90) and many pathological conditions (145,146). Glycoprotein storage diseases (e.g.α-mannosidosis, L-fucosidosis and sialosidosis), the reflection of genetic deficiencies of specific lysosomal hydrolases were studied with labelled lectin (57, 147). The data indicated that the lectin histochemistry provide a reliable specific diagnostic pattern for these glycoprotein storage diseases.

Many coupled lectins were used for cell surface glycoprotein studies. The intramembraneous deposits of

N-acetylglucosamine in glomerulonephritis was observed by fluorescein isothiocyanate (FITC)-labelled lectins (148). The selective binding of lectins could help in classification of renal diseases (149, 150). The cell surface of zoospores and zysts of fungus Phytophthora cinnamomi was also studied by the pattern of FITC-lectin binding (151). The model of nematode chemoreceptors on the head region of several nematode species based on the membrane glycoproteins were studied by lectin-mediated modification of chemotaxis (135). Chinese hamster ovary cell surface were distinguished between the normal and mutant cells in studying through surface sialic acids by WGA binding pattern (152). The data indicated that removal of surface sialic acids residues via neuramimidase treatment, resulting in a reduced ability to bind WGA. It is known that sialic acids play many important biological roles of cell surface activity. The localization study of this sugar has been attempted. At present, sialic acid binding lectin, LPA, conjugated to fluorescent marker or peroxidase are powerful probes for direct visualizations of sialoglycoconjugates (153, 154). The biochemical findings suggest that the loss or decrease in sialic acids content in tissue glycoconjugates is associated with a number of pathological conditions.

It is well known that malignant cells lose their contact inhibition or cell recognition. It was pointed out that the alternation of cell surface component;

glycoproteins and glycolipids, may occur. Changes in sugar content give advantages for lectin binding study. In recent years, many research groups have begun to take advantage of the alternation of cell surface glycoconjugates and selectively binding for an analysis of differentiation and transformation of cells in different biological system (155, 156, 138). The structure of lectin receptors on human mammary carcinomas were observed with conjugate lectins by both electron and light microscopy (139, 157). The results indicated that peroxidase-conjugated lectins (e.g. peanut lectin (PNA), Helix pomatia or soybean lectin) selectively reacted with those carcinoma and the presence of lectin receptors in this tumor as marker is more complex. Some investigators found with light microscope, that in differentiated mammary carcinoma PNA receptors were located mainly on the luminal cytoplasmic membrane, undifferentiated mammary tumor had few or no receptors in contrast to that finding in electron microscopy. It was also found that there was significant relationship between PNA binding with neuraminidase treatment and tumor differentiation (158).

Colorectal cancer has been intensively studied in terms of the morphology of mucosa, secretive and epithelial antigen (CEA) changes, since gastrointestinal mucins are glycoproteins (59). Changes in their composition have long been known to occur with cellular differentiation and neoplastic transformation. The lectin binding pattern would help in identification of the high risk population.

Besides, the amount of enzymes or fluorescein coupled lectin in the cytological diagnosis of malignancy, lectins may be of further used for the detection of glycoproteins shed from tumor cells by means of enzyme-linked lectin assay (ELLA) (160), this technique is modified from enzyme-linked immunosorbent assay (ELISA). It can be used in quantitative determination of soluble glycoconjugates of lectin receptors (161). It is hoped to be of advantage in analysis of the cancer soluble antigens by using various lectin probes.

I.3 Problem of the Study

It is known that cancer is a fatal disease that has not been completely curable yet. An early detection is one of the best approaches for the control of this disease. Several clinical and laboratory methods for early detection have been established and employed. A search for new, simple, rapid, economical and as well as sensitive methods is still being made. Assay for tumor marker is one of the methods of early detection. It is also advantageous in diagnosis, follow up and prognosis of cancer patients. Among the tumor markers have been reported, sialoglycoconjugates are an important and interesting one. At present, the determination of sialoglycoconjugates has been done by chemical methods with many technical limitations. Total sialic acids are usually measured as the free form after hydrolysis from their lipid- or protein-bound molecules. By acid hydrolysis and

colorimetry, no exact information about the intact molecules or their derivatives can be obtained. Thus, alternative specific biological methods are required and searched for. Recently, different lectins and labelled lectins have been introduced to be used in histopathology for the identification, characterization and semiquantitation of sialoglycoconjugates. In this study, an experimental attempt was made to apply sialic acid-binding lectins : wheat germ lectin (WGA), horse shoe crab lectin and other plant lectins, including influenza viral hemagglutinin for the characterization and estimation of sialoglycoconjugates in serum and tissue section samples from cancer patients.

I.4 Objectives

1. To isolate and partially purify the following plants and animal lectins :-

: wheat germ lectin from Triticum vulgaris
: horse shoe crab lectin from Tachypleus gigas
: lectin from garden pea seed Pisum sativum
: lectin from lens culinaris

The lectin preparations were used in hemagglutination-inhibition (HAI) assay for serum sialoglycoproteins by microplate titration method.

2. To perform hemagglutination inhibition assay using the prepared lectins and influenza viruses for a semiquantitative estimation of sialoglycoproteins in serum

samples from normal and cancer patients. Their HAI titers^{-1} obtained will be related to sialic acids content and the disease conditions.

3. To prepare the peroxidase-lectin conjugates which were further used to detect sialoglycoproteins present in tumor tissues by histochemical staining and microscopy.