CHAPTER 1

INTRODUCTION

1.1 Definition of lectin

Defining the term lectin encounters some difficulties. The adoption of one set of criteria will include some but not other molecules which have at one time been considered as lectins. The word lectin was derived from the latin word "legere" which means to choose or to pick out. This was proposed by Boyd and Shapeigh (1954) based on the observation of selective agglutination of erythrocytes from human blood groups by some lectins. The study of lectin was initiated by Stillmark, who discovered the phenomenon of hemeagglutination by extracts of caster beans in 1888. Since then a variety of plants and living things were reported to contain such lectins. After that, the original definition of lectin has to be broadened as lectins are not only found in plants but also in slime molds bacteria, sponges, invertebrates and vertebrates (Lis & sharon, 1977). A broader lectin definition suggested by Goldstein *et al* (1980) stated that lectins are carbohydrate binding proteins of nonimmune origin which can agglutinate cells and/or precipitates glycoconjugates. These substances bear at least two binding sites, may be soluble or membrane bound and are either of bacterial or plant origin. This definition allows inclusion of non-agglutinating lectins, insoluble lectins in biological fluid but make no distinction between lectins and enzymes of sugar metabolism. Any sugar-specific enzymes with more than one binding sites such as glycosidase, glycotransferase, can act as lectin. Ricin and abrin, two toxins which bind to the

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surface of erythrocytes but are monomeric, possess only one binding site and cannot agglutinate cells are excluded under this definition. This definition has been modified with several transitions as information about these molecules have accumulated. The latest definition was suggested by Kocourek and Horejsi (1981) and is based on the physicochemical properties of the interaction with carbohydrates. Under this definition, the toxin of *Ricinus communis* (ricin) and *Abrus precatorious* (abrin) are lectins. Kocourek and Horejsi defined that lectins are sugar-binding proteins or glycoproteins of nonimmune origin which are devoid of enzymatic activities toward sugars to which they bind and do not require free glycosidic hydroxyl group on these sugars on their binding. This latest definition is now more accepted.

Since the widely used method of lectin identification is cell agglutination, lectin is also called agglutinin. The difference between the words agglutinin and lectin is that the former includes all cell agglutinable substances while the latter represents cell agglutinable substances with sugar specificity.

1.2 General properties of lectins

In their interaction with cells, lectins bind readily to cell surface resulting in biological effects. The binding leads to cell agglutination and changes in their cellular properties. In addition, the interaction can be specifically inhibited by sugars. This section will deal with the general properties of lectins.

1.2.1 Cell agglutination

Agglutination is the easiest manifestation of lectin interaction with cells, to this day it is used to reveal the presence of a lectin in a biological source. Cell agglutinating property distinguishes lectins from other sugar binding molecules such as glycosidases and glycotransferases.

When agglutination occurs, the bound lectins must form multiple cross-bridges between opposing cells. However, it is difficult to determine the actual amount of lectin bound to cells. Some lectin cannot agglutinate cells unless the cells are treated with mild hydrolytic enzymes. For example, 1 mg/ml of peanut agglutinin, which was considered to be a high concentration, could not agglutinate erythrocytes unless the cells were treated with sialidase (Lis & Sharon, 1981). It was suggested that the enzymatic treatment does not affect the total number of lectin binding sites (Sharon & Lis,1975), but may cause a decrease in negative charges and the removal of peptides and glycoproteins from the cell surface that may hinder the agglutination (Shnebli *et al*, 1976).

Some lectins require metal ions for binding activity ; for example, soybean agglutinin from *Glycine max* requires Mn^{2+} and Ca^{2+} for hemagglutinating activity and results in 10 and 15% increment ,respectively (Jaffe *et al*,1977).

There are various factors affecting cell agglutination by lectins ; for example , properties of lectins such as number of saccharide-binding site, strength of saccharide binding, electrical charges or molecular size. Cell surface properties such as chemical structure, surface rigidity, electrical charges or number and distribution of receptor sites also play important role (Lis &

Sharon, 1977). In addition, agglutination is affected by external conditions such as temperature and pH. For instance, Con A is a poor agglutinin at 4°C whereas at 37°C it is a strong agglutinin. This effect has been discussed in term of molecular form of this lectin which forms a dimer at 4°C but a tetramer at 37°C (Lis & Sharon, 1977). Effect of pH on the binding of lectin from *Vicia graminea* to erythrocytes has been reported by Duk and Lisowska (1984) that decreased protonation of amino acids on the receptor enhanced binding of the lectin to human erythrocytes at pH 6-8. However,it was observed that at pH 10 the binding of lectin was lowered by 80% of maximal values.

Most studies on agglutination of cells by lectins have been carried out with animal cells. Other cells such as bacteria, fungi or viruses and subcellular particles such as nuclei and mitocondria can also be agglutinated by lectins (Lis & Sharon,1986).

1.2.2 Specificity of carbohydrate-binding to lectins

It was concluded that agglutinating properties of the lectins resulted in part from the presence of specific carbohydrate binding sites on these molecules (Sharon & Lis, 1972). This conclusion was proved by inhibition of cell agglutination by appropriate sugar(s). It can be taken as an indication that similar sugar(s) is present on the surface of the cells agglutinated by the lectin.

Sugar-lectin complementarity is determined generally by the hepten inhibition technique Lansteiner (1962) on the basis of the minimal concentrations of sugars required to inhibit the hemagglutination reaction or the precipitin reaction between the lectin and reacting molecule.

Lectins can be classified into a rather limited number of carbohydrate-binding groups based on their preferential binding to D-pyranose ring. These include the mannose/glucose binding lectins, N-acetyl-galactosamine/galactose binding lectins , the L-fucose binding lectins, sialic acid-binding lectins, and those with complex binding sites.

While binding site of some lectins appears to be complementary to a single glycosyl unit, many others have been found to possess extended binding site accommodating two to five or six sugar residues. For example, wheat germ agglutinin and the solanaceae lectin (potato, tomato, *Datura stramonium*) interact most strongly with β-(1,4)-linked N-acetyl-glucosamine oligomers. Peanut agglutinin binds preferentially to Gal β-(1,3)-GalNac unit (Wu,1984), whereas tri- and tetra- saccharides were better inhibitors of marrow (*Cucurbita pepo*), cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) agglutinin than disaccharides (Anthony,1979).

Interactions of lectins to sugars differ markedly with respect to their anomeric specificity when interact with reducing terminal glycosyl group of the polysaccharide chain. Con A and pea lectins exhibit anomeric specificity whereas other lectins, such as soybean and caster bean appear to be almost indifferent.

With few exceptions, lectins interact with nonreducing terminal glycosyl groups at the end of the polysaccharide and glycoprotein. Some binds to the internal sugar unit of the polysaccharide chain. For example, marrow lectin and wheat germ agglutinin interact with internal disaccharides (Anthony, 1979).

Beside the carbohydrate ligands, binding of non-carbohydrate ligands has been observed for several lectins. For example, Con A and a large series of legume and nonleguminous lectins

have been shown to bind fluorescent hydrophobic molecules such as ANS (1,8-anilinonaphthaline sulfuric acid) and TNS (2,6-toluidinylnaphthalene sulfuric acid) (Robert & Goldstein, 1982). This implies the presence of hydrophobic binding site in the lectin molecules.

The ability of some lectins to bind carbohydrate and noncarbohydrate ligands is of paramount importance when considering lectin functions and applications.

1.2.3 Other biological properties of lectins

Other properties of lectins are summerized in Table 1 by Lis & Sharon (1986). Most of them result in irregular changes of cells when lectins bind to the cell-surface sugars.

1.3 Distribution of lectins

Lectins have been found, with varieties of sugar specificity , in many living things such as microorganisms , invertebrates, algae, protozoa, slime molds and higher animals including plants (Kocourek, 1986; Sharon & Lis,1989; Zatta & Cumming 1992).

Virus hemagglutinin was first found in 1941 by Hirst who observed that human influenza virus can agglutinate erythrocytes and other cells. It was later shown that the virus recognized N-acetylneuraminic acid, one of the sialic acid present on the cell surface of erythrocytes or other cells and the binding is a prerequisite for initiation of infection (Paulson,1987).

In Bacteria , many enterobacteriaceae have lectins in their pilli that are essential for their successful adhesion to the intestinal epithelium leading to infection in urinary and

Table 1 Biological properties of lectins (Lis & Sharon, 1986)

Properties of lectins
Agglutination of erythrocytes and other types of cell
Mitogenic stimulation of lymphocytes
Generation of suppressor cells
Mediation of killing of target cells by lymphocytes and macrophages
Enhancement of phagocytosis of yeast and bacteria by macrophages
Insulin-like effects on fat cells
Toxicity to cells and animals
Inhibition of growth and tumor cells
Induction of vacuole formation and macrophages
Immunosuppresive effect *in vivo*
Promotion of cell adhesion and spreading
Induction of platelets release reaction
Induction of histamine release from basophils and mast cells
Induction of patching and capping of receptors on cell surfaces
Peroxide release from macrophages
Induction of endocytosis of lysozomal enzyme by cultured fibroblasts
Inhibition of fungal growth

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gastrointestinal tract. For example, both *Escherichia coli* and *Samonella* spp. have several lectins with different carbohydrate-binding specificity. Because of their apparent association with other proteins in pilli, these lectins were found difficult to purify and characterize (Zatta & Cumming, 1992).

Lectin was also found in protozoa ; *Entamoeba histolytica*, a pathogenic amoeba which causes dysentary in human by disruption and invasion of colonic mucosa. Various saccharides were found to inhibit amoebic adherance to enterocytes and other mammalian target cells *in vitro*. It was suggested that lectins may mediate binding site of the parasite to other cells and tissues, particularly to hepatocytes , initiating the killing of these cells (Sharon & Lis, 1989).

Discoidin I, a galactose-specific lectin in the slime mold, *Dictyostelium discoideum*, was suggested to play an important role for cell-cell aggregation in its differentiation stage (Barondes, 1984).

Invertebrates and vertebrates were also found to contain lectins both in intracellular and extracellular parts,such as in hemolymph of Limunus (the horseshoe crab) and in sexual organs of some mollusca and arthopoda. Higher animals produce varieties of lectins both in membrane bound and soluble forms. The agglutinating properties and the sugar specificities implicate the function of lectins in these organisms. For example, cell recognition phenomena of galactose specific membrane bound lectin isolated from rat peritoneal macrophages, displayed the recognition and phagocytosis events of *Trypsinoma cruzi*, a parasite causing Chagas' disease (Sharon and Lis,1989).

Plant is the first source lectin was discovered and most

frequently used in lectin studies due to the ease of extraction and the yeild obtained. Lectins can be found widespread throughout the plant kingdom in different families of plant as well as different tissues in the same plant which contain lectins with different molecular properties and variety of carbohydrate specificities. It was suggested that plant lectins may have important roles according to their abundance. Lectins may have been adapted for several functions during evolution (Etzler,1985).

1.4 Functions of plant lectins

Although speculations have been made, little is known about the actaul functions of plant lectins. Many hypotheses have been proposed, most extensive suggestions are the roles of lectins in : a) mediation of symbiosis in nitrogen fixation b) protection of plant against pathogens.

There are several indirect evidences supporting the role of lectins in establising symbiosis between rhizobium and leguminous plant which leads to nitrogen fixation process. These evidences either showed the present of lectins and their appropriate receptors at the same time and place which nodulation by the bacteria occurs; or the ability of the host lectin to recognize specific nodulating strain of rhizobium. Some lectins such as soybean agglutinin, trifoliin, Con A and pea lectin were shown to particularly agglutinate bacteria from rhizobium species (Pistole, 1981) but only trifoliin of white clover can bind a specific nodulating strain of rhizobium. This lectin has been suggested to reversibly form cross bridge receptors between the cell wall of root hair and bacterial capsular polysaccharides and/or lipopolysaccharides as a prelude

to nodolation (Wolpert & Albersheim,1976). In addition, there was evidence of association between diazotrophs *Klebsialla* spp. with rice root by the adhesion function of rice lectin and bacteria (Boonjawat *et al*, 1991)

The role of lectin in defense mechanism of plants may have evolved from the ability of lectins to agglutinate and immobilized bacteria or other microorganisms. Etzler (1985) summarized the supporting evidences for this proposed role into two main observed actions ; a) a presence of lectin at the potential sites of invasion by infectious agent (Fountain *et al*,1977), b) the binding of lectins to various fungi and their ability to inhibit fungal growth and germination (Mirelman *et al*,1975).

A number of study with respect to plant defense role of lectins have been reported. For example, during the imbibition of dry soybean seeds, lectin is released into water. The presence of lectin in the vicinity of germinating seed hint possible interaction of lectin with potential pathogen (Fountain *et al*,1977). The developmental pattern of initial accumulation and final disappearance of lectin can be observed during the seed dormancy, germination, and maturation. This evidence may implicate the role of lectin in a defense mechanism necessary for plant in the initial stage of growth (Howard *et al*, 1972).

Mirelman *et al* (1975) reported the inhibitory effect on growth of *Tricoderma viride* by purified wheat germ agglutinin at the site of hyphal tips and septa. Eleven purified seed lectins which were representatives of five groups of sugar-specificity can disrupt the growth of germinating fungal spore by binding to germ tube and cause sensitivity to osmotic lysis. Similar result has been obtained from the chitin-binding lectin from rhizome of stinging

nettle (*Urdica dioica* L.) which caused hyphal lysis to the fungi (Broekaert,1989).

Another interesting finding of surface lectin in cucumber(*Cucumis sativus*) seedling was reported and its role suggested (Kessler, 1988). This lectin was extracted from cotyledon surface by simply immersing cotyledon in water. The extract exhibited high specificity for chitin and chitosan, was stable both in broad pH range (3-10) and high temperature up to 85°C and was resistant to proteolytic enzymes. In addition, this extract displayed peroxidase and superoxide dismutase-like activities, a system for detoxification of superoxide and oxygen derived free radicals. Besides, it can inhibit formation of conidio spores of powdery mildew , necrotic spots of tobacco necrotic virus (TNV) and spore germination of some fungi. These evidences implicate the prevention and protection from the invasion of pathogens and extreme environmental conditions (Kessler, 1988).

Most of the lectins which were proposed with a role in plant defense mechanisms are observed to be specific to chitin or N-acetyl-D-glucosamine, its monomer. This correlates well with its observed antifungal growth effect as chitin is common wall constituent of fungi and bacteria. Lectins have also been implicated as a defensive compound against certain insect pest. Murdork and co-worker (1990) had studied on the biological effects of some plant lectins on cowpea weevil (*Callosobrucus maculatus*). Using artificial seed system, it was found that the in presence of peanut agglutinin the developmental time of cowpea weevil was delayed by 0.49 days for every 0.1% increment and 1.47 days for every 0.1% increment in presence of WGA. Inhibitory effect of WGA on development of two important maize pests, the European corn borer (*Ostrinia nubilalis*) and southern

corn rootworm (*Diabrotica undecimpurictata*) was also observed *in vitro* (Czapla and Lang, 1991).

In contrast, there is a suggestion that lectin may aid the invasion of plant by some pathogens by serving as receptors for phytotoxin or facilitate the attachment of the pathogen such as in the case of infection of sugar cane by the fungus *Helminthosporium sacchari*. The fungus produces a toxin which bind to a protein on plasma membrane of sugar cane leaves. This protein has lectin-like characteristics in that its binding to the toxin is specifically inhibited by galactosides and its multivalent (Etzler, 1985).

Other roles of plant lectins have been proposed including their functions in cell wall extension, cell wall recognition, growth regulation, carbohydrate transport and storage. Several of these roles are consistent with the current states of information on lectin localization and continued to be explored (Lis & Sharon, 1981).

1.5 Application of lectins

A discovery of a large number of lectins with different carbohydrate specificities have led to their extensive utilization as biological tools for study of simple and complex carbohydrates in solution and on cell surface, for identification and separation of cells, including histochemical and cytochemical study.

Lectins serve as purification tool when immobilized on a solid matrix such as agarose as affinity column. Cell or tissue derived glycoproteins can be passed through the column and only specific carbohydrate structure recognizes and binds to the lectin ligand. The glycoproteins can be released from the immobilized lectins by elution with appropriate concentration of competing sugar or carbohydrate in

one step or using a concentration gradient. Using this type of procedure, many glycoproteins , glycolipids and glycosylated compounds have now been purified ; for example, receptor for insulin, and epidermal growth factor (Zatta & Cumming ,1992 and Lis & Sharon, 1985). Detection of subpopulations of mammalian , β-adrenergic receptor, one containing oligomannose and other containing complex type carbohydrate was achieved by Con A and WGA affinity chromatography (Lis & Sharon,1985). Interaction of peanut agglutinin with immature thymocytes by selective agglutination and affinity chromatography have been successfully applied to isolate different cells (Lis & Sharon,1981).

Identification of glycoproteins in solutions can be done on polyacrylamide gel electrophoresis by staining with radioactive or fluorescent labeled lectins. Another useful procedure is affinity immunoelectrophoresis. Glycoproteins can be separated by conventional electrophoresis using lectin as the first identification probe followed by lectin antibody as the second probe (Zatta & Cumming,1992).

Lectins can be used as histochemical and cytochemical probes to study carbohydrate changes, alteration of cell membrane, including glycosylation process and detection of cell abnormality in cell differentiation (Etzler,1985). Glycoprotein storage disease can be detected by principle of histochemistry in cooperation with lectin, *e.g.* using lectin for probing undegraded oligosaccharides on paraffin-embedded tissue sections. This can reduce time consumption when comparison with detection of those oligosaccharides by demonstrating a deficiency in carbohydrate degradable enzyme activity or an elevation of undegraded oligosaccharides in cells or body fluid (Alroy *et al*, 1984).

The use of lectin in microbiology is still based on binding of

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lectins to different carbohydrate structure on surface of microorganisms ; thus, lectins are useful in laboratory identification of microorganisms. For instance, potato lectin specifically agglutinates *Pseudomonas solanacearum* (Allen *et al*, 1978) and Con A agglutinates *Entamoeba histolytica* (Palmo & Robels,1973). Under the same principle , one of the earliest application of lectins and is still widely used is in distinguishing erythrocytes and blood groups. Several lectins are specific to blood types A,B,O and other groups. For example, lectin from *Lotus tetragonolobus* serves to identify O-cells (Lis & Sharon, 1986).

Another useful application of lectin is adapted from the ability of some lectins to interact preferentially with certain transformed cells, leading to the attempt to use this compound as carrier for chemotherapeutic agents. For example, conjugation of Con A with antitumor drugs such as chlorambucil (Lin *et al*, 1981) and metrotexate (Lin *et al*,1981 , Tsumo *et al*,1980) led to more effective action on various cultured tumor cell lines in comparison with equivalent doses of free drug and lectin *in vitro*. However, no evidence is available *in vivo*.

The novel application of lectins in agriculture is on going. In account of the possible functions of plant lectins in rhizobium-legume symbiosis or plant protection against pathogenic organisms as mentioned earlier, better understanding at molecular biology level of plant lectins is still required. Reseaches on plant-microbe interaction have been attempted to discover the nature of this interaction, with the hope of being able to apply the knowledge in field scale in protecting crops against diseases and increasing crop yield and quality. For example, the gene of *Urdica dioica* agglutinin, a lectin from stinging nettle rhizome with antifungal

property is hoped to be isolated and inserted into the genome of the fungal susceptible plant (Broekaert *et al*,1989).

Recently, the presence of a lectin in cucumber (*Cucumis sativus*) on the epicuticular surface has been reported (Kessler,1988). Some of the reported properties suggested its possible biological function in plant defense reaction as mentioned earlier. Correlation of its presence on the surface of cotyledon seedlings at certain developmental stage and its antifungal effect indicates its involvement in protection against plant pathogens.

Angled loofah (*Luffa acutanggula* Roxb.), a local plant in Thailand, is one of cucurbit plant in the family Cucurbitaceae. It is an annual crop plant which can be grown in high humidity condition with whole day light in temperature range 20-30°C in all kind of soil (กลุ่มหนังสือเกษตร,2525). Many pathogens can cause damage in cucurbit plants ; for example, powdery mildew wilt , antracnose and downy mildew. It was found that loofah has good resistance to antracnose and downy mildew (ดนงค์ ,2528). Preliminary screening of a few local cucurbit plants showed that pericarp of angled loofah has high hemagglutinating activity (รัติยา ,2530). Thus, lectin in angled loofah may also play role in defense mechanism against pathogens. The general knowledge of angled loofah, the preliminary screening in combination to the report of Kessler (1988) led to the idea of this research work.

The aims of this thesis are

1) To purify and charaterize lectin from fruit pericarp of *Luffa acutangula* Roxb.

2) To identify lectin on surface of organs of the angled loofah seedling and purify it.

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3) To comparatively study some of their biological properties on plant defense reaction.

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