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Lectins: production and practical applications

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Abstract Lectins are proteins found in a diversity of organisms. They possess the ability to agglutinate erythrocytes with known carbohydrate specificity since they have at least one non-catalytic domain that binds reversibly to specific monosaccharides or oligosaccharides. This article aims to review the production and practical applications of lectins. Lectins are isolated from their natural sources by chromatographic procedures or produced by recombinant DNA technology. The yields of animal lectins are usually low compared with the yields of plant lectins such as legume lectins. Lectins manifest a diversity of activities including antitumor, immunomodulatory, antifungal, HIV-1 reverse transcriptase inhibitory, and anti-insect activities, which may find practical applications. A small number of lectins demonstrate antibacterial and anti-nematode activities.

Keywords Lectins · Hemagglutinins · Production · Practical applications

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Introduction

“Lectin” comes from the Latin word “legere”, which means “to select”, by William Boyd in 1954. Lectins have the ability to bind carbohydrates. Nowadays, proteins that can agglutinate red blood cells with known sugar specificity are referred to as “lectins”. The name “hemagglutinins” is used when the sugar specificity is unknown.

Lectins and hemagglutinins are proteins/glycoproteins, which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharides or oligosaccharides. They can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes, without altering the properties of the carbohydrates.

Lectins with specific carbohydrate specificity have been purified from various plant tissues and other organisms. They can be classified on the basis of their carbohydrate specificity. They can also be categorized according to the overall structures into merolectins, hololectins, chimerlectins and superlectins, or be grouped into different families (legume lectins, type II ribosome-inactivating proteins, monocot mannose-binding lectins, and other lectins).

The content of lectin varies in different organisms. The high yields of lectins from different sources may facilitate mass production. Application of lectins is possible depending on their properties. The antifungal and anti-insect activities of lectins can be made use of in the control of pathogens. The production of anti-tumor and anti-viral drugs based on lectins may also be feasible.

Production of lectins

Lectins are found in nature. A large number of lectins or hemagglutinins have been purified from different organisms.

On the other hand, lectins can be produced by recombinant techniques.

Recombinant lectins

Lectins can be produced by recombinant techniques. The natural sources and yields of some lectins are summarized in Table 1. The yields of lectins were very low (0.1–5 mg/L culture medium) before the advent of the nineteenth century. Recently, the yield attained is higher (up to 20 mg/L culture medium).

In order to produce a sizeable quantity of a lectin, e.g., 10 g, large-scale fermentation is the only way. However, a large fermentor in GMP condition is required. The cost of fermentation is high. *Escherichia coli* is the most popular expression system used. Different strains, e.g., BL21 (DE3) RIL strain and Nova Blue (DE3) strain, were chosen for expression of different lectins. In general, *E. coli* transformed with expression vectors is grown in sterilized culture medium, e.g., BMGY medium and Luria-Bertani medium, in a fermentor. Antibiotics, e.g., ampicillin, are added to reduce the chances of contamination. Fermentation is carried out in defined pH value, temperature, optical density at the time of induction, inducer concentration and time of expression. Isopropyl β -D-thiogalactoside is the most frequently used inducer. After induction, the cells are harvested by centrifugation. Lectins are released from the cells by resuspension in lysis buffer followed by sonication. Further chromatographic steps are required for purification of lectins (Upadhyay et al. 2010; Tateno et al. 2004).

Natural lectins

Animal lectins

Lectins are found in different animals. However, the yields are usually extremely low (Table 2). Mass purification of animal lectins necessitates bulk quantities of raw materials which make it not feasible.

Mushroom lectins

Yields of lectins from fresh mushrooms are low, e.g., 2.6 mg from 100 g of fresh fruiting bodies of *Pleurocybella porrigens* (Suzuki et al. 2009). In fact, the water content in fresh mushrooms is very high. Dried fruiting bodies of the mushrooms *Russula lepida*, *Pholiota adiposa*, and *Inocybe umbrinella* yielded 39, 70, and 15 mg lectin per 100 g fruiting bodies, respectively (Zhang et al. 2010, 2009; Zhao et al. 2009). Therefore, production from fresh mushroom is also unpractical.

Plant lectins

The lectin contents in some parts of plants are higher, e.g., 390 and 75 mg of the purified lectin was recovered from 100 g *Remusatia vivipara* tubers (Bhat et al. 2010) and *Astragalus mongholicus* roots (Yan et al. 2005), respectively. Lectins are also found in seeds. The lectin content in non-legume plants is low, e.g., 3.3 mg lectin from 100 g *Hibiscus mutabilis* seeds (Lam and Ng 2009). Lectins are found in abundance in legume seeds. *Phaseolus vulgaris* is an herbaceous annual plant grown worldwide for its edible

Table 1 Yields of plant lectins produced by recombinant DNA techniques

Natural source of lectin	Lectin yield (mg/L culture medium)	Genetically modification in cells	Reference
<i>Allium sativum</i> (garlic) leaf	5	cDNA was cloned into NdeI and BamHI restricted plasmid pET19b and expressed in <i>E. coli</i> strain BL21 (DE3) cells	Upadhyay et al. 2010
<i>Artocarpus incise</i> (breadfruit)	16	cDNA was cloned into the pET-25b(+) and expressed in <i>E. coli</i> .	Oliveira et al. 2009
<i>Artocarpus incise</i> (breadfruit)	18–20	cDNA was cloned into EcoRI/XbaI restricted plasmid pUC57 and expressed in <i>E. Coli</i>	Oliveira et al. 2008
<i>Glycine max</i> (Soybean)	0.1	cDNA was cloned NcoI/NdeI/BamHI restricted plasmid PET-3d and expressed in <i>E. coli</i> strain BL21(DE3)pLysS	Adar et al. 1997
<i>Nicotiana tabacum</i> (tobacco) leaves	6	cDNA was cloned EcoRI/NotI restricted plasmid and expressed in <i>E. coli</i> strain top10F	Lannoo et al. 2007
<i>Oryza sativa</i> (rice) roots	14.6	cDNA was cloned into NdeI/BamHI restricted pET 3D plasmid and expressed in <i>E. coli</i> strain BL21 (DE3) cells	Branco et al. 2004
<i>Pisum sativum</i> (pea)	2–5	cDNA was cloned into HindIII/PstI/BamHI restricted plasmid and expressed in <i>E. coli</i> strain W3110	Stubbs et al. 1986
<i>Polyporus squamosus</i> fruiting bodies	4–7	cDNA was cloned into NdeI/BamHI restricted plasmid and expressed in <i>E. coli</i> strain Nova Blue (DE3)	Tateno et al. 2004

Table 2 Yields of animal lectins obtained by chromatographic isolation from natural sources

Natural source	Chromatography for purification	Lectin yield	Reference
<i>Acropora millepora</i> (coral) plasma fluid	Mannose affinity chromatography	0.7 mg/100 ml plasma	Kvennefors et al. 2008
<i>Aristichthys nobilis</i> (bighead carp) gills	DEAE-Sepharose, Sephacryl S-200 and Superdex 200	9.4 mg/100 g	Pan et al. 2010
<i>Bubalus bubalis</i> (Buffalo) heart tissue	Ammonium sulfate precipitation and Sephadex G50	0.97 mg/100 g	Ashraf et al. 2010
<i>Holothuria scabra</i> (sea cucumber) coelomic fluid	Ultrafiltration and Phenyl-Sepharose	1.6 mg/100 ml	Gowda et al. 2008
<i>Macoma birmanica</i> (marine bivalve) foot muscles	Ammonium sulfate precipitation and N-acetylglucosamine Sepharose 4B	4.5 mg/100 g	Adhya et al. 2009
<i>Nemipilema nomurai</i> (jellyfish)	SP-Sepharose and BSM-Toyopearl	0.35 µg/100 g	Imamichi and Yokoyama 2010

beans, popular in both dry and green bean forms. The commercial production of beans is well distributed worldwide. There are different varieties, including anasazi bean, black beans, cranberry bean, borlotti beans, pink beans, pinto beans, kidney beans, shell beans, white beans, yellow beans and French beans, etc. Lectins or hemagglutinins have been purified from different varieties of *P. vulgaris*. The lectin contents are low in some varieties and high in other varieties (Table 3).

Purification of lectins or hemagglutinins

Isolation of lectins can be achieved by a combination of different purification techniques. Acids (e.g., acetic acid used by Naeem et al. 2007), organic solvent (e.g., acetone used by Medeiros et al. 2010) or salt (e.g., ammonium sulfate) can be used to precipitate lectins. Various chromatographic methods, including affinity chromatography, ionic exchange chromatography, hydrophobic interaction

chromatography, and gel filtration can be utilized. An increase in the number of purification steps usually results in a lower recovery.

In order to produce a large quantity of lectins, the first criterion is a high lectin content in the starting material. The second criterion is the use of a simple purification protocol. Tetrameric *escumite* lectin was purified by affinity chromatography on a column containing glutaraldehyde membranes from blood group O erythrocytes. Four isoforms were separated on Mono-S (cation exchanger) (Castillo-Villanueva et al. 2007). Dark red kidney bean hemagglutinin was unadsorbed on DEAE-cellulose but adsorbed on Affi-gel blue gel (Xia and Ng 2006). French bean 35 hemagglutinin with high purity was isolated by chromatography on Blue-Sepharose and Q-Sepharose (Lam and Ng 2010b).

Notwithstanding recombinant lectins can be synthesized, the high cost and low yield are the drawbacks. From the retrospective reports, production of lectins very often relies on chromatographic isolation from plant sources. This is especially so for *Phaseolus* cultivars.

Table 3 Yields of plant lectins obtained by chromatographic isolation from seeds of different *Phaseolus* cultivars

<i>Phaseolus</i> cultivar	Chromatography for purification	Yield (mg/100 g seed)	Sugar specificity	Reference
Anasazi bean	Affi-gel blue gel, Mono S and Superdex 200	13	Not found	Sharma et al. 2009
Dark red kidney bean	DEAE-cellulose and Affi-gel blue gel	107	Not found	Xia and Ng 2006
Escumite bean	Affinity chromatography (glutaraldehydized membranes from blood group O erythrocytes)	163 (total of 4 isoforms)	N-acetyllactosamine-type glycans	Castillo-Villanueva et al. 2007
Extralong autumn purple bean	Blue-Sepharose, Q-Sepharose, Mono Q and Superdex 75	35	Galactose	Fang et al. 2010
French bean 12	SP-Sepharose, Affi-gel blue, Q-Sepharose, and Superdex 200	4.8	Not found	Leung et al. 2008
French bean 35	Blue-Sepharose, Q-Sepharose and Superdex 75	1100	Not found	Lam and Ng 2010b
Red kidney bean	Affi-gel blue gel and CM-Sepharose	27.5	Lactoferrin, ovalbumin, thyroglobulin	Ye et al. 2001

Practical applications

Lectins–pathogen management strategies

Anti-insect activity of lectins

Lectins have been suggested as one of the promising agents against insect pests and have been engineered successfully into a variety of crops including wheat, rice, tobacco, and potatoes. This approach could be used as a part of integrated pest management strategies and caveat pest attack.

In general, it seems that large-scale implementation of transgenic insecticidal and herbicide-tolerant plants does not display considerable negative effects on the environment. Moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerably reduces the load of chemical insecticides and herbicides (Velkov et al. 2005).

Lectins demonstrate anti-insect activity. They increase the mortality or delay the development of insect (Table 4). When incorporated in an artificial diet, *Arisaema jacquemontii* lectin adversely affected the development of *Bactrocera cucurbitae* larvae (Kaur et al. 2006a). *Arisaema helleborifolium* lectin exhibited anti-insect activity towards the second instar larvae of *B. cucurbitae* (Kaur et al. 2006b). The insecticidal property of lectins may be due to orchestration of enzymatic activity of larvae. After treatment with different lectins, the activity of esterases in larvae was increased whereas the activity of acid phosphatase and alkaline phosphatase decreased.

Galectin-1 treatment of *Plutella xylostella* larvae brought about disruption of the microvilli and induced abnormalities in these epithelial cells (Chen et al. 2009b). *Dioscorea batatas* lectin inhibited the emergence of *Helicoverpa armigera* larvae into adults by avidly binding to larval brush border and peritrophic membrane (Ohizumi et al.

2009). *Arum maculatum* tuber lectin caused *Lipaphis erysimi* and *Aphis craccivora* to succumb, by binding to the gut brush border membrane vesicle proteins (Majumder et al. 2005). *Olneya tesota* lectin bound to midgut glycoconjugates and microvillae of *Zabrotes subfasciatus* larvae. Diminished oviposition and a failure of emergence of adult beetles were observed (Lagarda-Diaz et al. 2009). *Annona coriacea* lectin displayed toxicity in *Anagasta kuehniella* which apparently resulted from a change in the gut membrane environment and consequent disruption of digestive enzyme recycling mechanisms by binding to midgut proteins (Coelho et al. 2007). *Bauhinia monandra* leaf lectin produced mortality in *Zabrotes subfasciatus* and *Callosobruchus maculatus* when incorporated into an artificial diet. *B. monandra* leaf lectin produced a 40% decrement in weight of *A. kuehniella* larvae. *B. monandra* leaf lectin bound to midgut proteins of the insect *C. maculatus* (Macedo et al. 2007).

The detached leaves from transgenic tobacco plants expressing *Allium sativum* lectins reduced the weight gain and development and the metamorphosis of *Spodoptera littoralis* larvae. Furthermore, the larvae were detrimental to the pupal stage resulting in weight reduction and lethal abnormalities (Sadeghi et al. 2008). Production of *Rhopalosiphum maidis* nymphs was significantly reduced on *Galanthus nivalis* agglutinin-expressing plants (Wang et al. 2005). *G. nivalis* agglutinin was also found bound to glycoproteins that can be found in the guts of larvae of *Adalia bipunctata*, *Chrysoperla carnea*, and *Coccinella septempunctata* (Hogervorst et al. 2006).

Antifungal activity of lectins

Despite the large numbers of lectins and hemagglutinins that have been purified, only a few of them manifested

Table 4 Anti-insect activity of lectins

Natural source of lectin	Insect affected	Anti-insect effect	Sugar specificity	Reference
<i>Allium sativum</i> (garlic) bulbs	<i>Acyrtosiphon pisum</i>	Increased mortality	Mannose	Fitches et al. 2008
<i>Arisaema intermedium</i> and <i>Arisaema wallichianum</i> (Araceae)	<i>Bactrocera cucurbitae</i>	(1) Prolonged period of development (2) Inhibited pupation and emergence	Not found	Kaur et al. 2009
<i>Gracilaria cornea</i> (red alga)	<i>Boophilus microplus</i>	Reduced (1) the body weight of female after oviposition period, (2) the egg mass weight, and (3) hatching period	Fetuin, porcine stomach mucin	Lima et al. 2005
<i>Gracilaria ornate</i> (red alga)	<i>Callosobruchus maculatus</i>	Delayed development	Fetuin, porcine stomach mucin	Leite et al. 2005
<i>Myracrodruon urundeuva</i> (aroeira preta) bark	<i>Aedes aegypti</i>	Increased mortality	N-acetyl-D-glucosamine	Sá et al. 2009
<i>Xerocomus chrysenteron</i> fruiting bodies	<i>Myzus persicae</i>	Increased mortality	Fetuin, porcine stomach mucin	Jaber et al. 2008
<i>Xerocomus chrysenteron</i> fruiting bodies	<i>Myzus persicae</i>	(1) Increased mortality (2) Reduction of body weight, duration of development and fecundity	Fetuin, porcine stomach mucin	Jaber et al. 2007

antifungal activity (Table 5). The expression of *Gastrodia elata* lectins in the vascular cells of roots and stems was strongly induced by the fungus *Trichoderma viride*, indicating that lectin is an important defense protein in plants (Sá et al. 2009). Following insertion of the precursor gene of stinging nettle isolectin I into tobacco, the germination of spores of *Botrytis cinerea*, *Colletotrichum lindemuthianum*, and *T. viride* was significantly reduced (Does et al. 1999). Thus, lectins may be introduced into plants to protect them from fungal attack.

Plant lectins can neither bind to glycoconjugates on the fungal membranes nor penetrate the cytoplasm owing to the cell wall barrier. It is not likely lectins directly inhibit fungal growth by modifying fungal membrane structure and/or permeability. However, there may be indirect effects produced by the binding of lectins to carbohydrates on the fungal cell wall surface. Chitinase-free chitin-binding stinging nettle (*Urtica dioica* lectin) impeded fungal growth. Cell wall synthesis was interrupted because of attenuated chitin synthesis and/or deposition (Van Parijs et al. 1991). The effects of nettle lectin on fungal cell wall and hyphal morphology suggest that the nettle lectin regulates endomycorrhizal colonization of the rhizomes. Several other plant lectins inhibit fungal growth. The first group includes small chitin-binding merolectins with

one chitin-binding domain, e.g., hevein from rubber tree latex (Van Parijs et al. 1991) and chitin-binding polypeptide from *Amaranthus caudatus* seeds (Broekaert et al. 1992). The only plant lectins that can be considered as fungicidal proteins are the chimerolectins belonging to the class I chitinases. However, the antifungal activity of these proteins is ascribed to their catalytic domain.

Lectins–antitumor drugs

It is well documented that lectins have an antitumor effect. *Flammulina velutipes* hemagglutinin-inhibited proliferation of leukemia L1210 cells (Ng et al. 2006). *Haliclona cratera* lectin displayed a cytotoxic effect on HeLa and FemX cells (Pajic et al. 2002). Dark red kidney bean hemagglutinin exerted an antiproliferative activity toward leukemia L1210 cells (Xia and Ng 2006). Small glossy black soybean (*Glycine max*) lectin impeded proliferation of breast cancer MCF7 cells and hepatoma HepG2 cells (Lin et al. 2008). Del Monte banana lectin retarded proliferation of (L1210) cells and hepatoma (HepG2) cells (Cheung et al. 2009). Extralong autumn purple bean lectin inhibited the proliferation of hepatoma HepG2 cells by inducing the production of apoptotic bodies (Fang et al. 2010). Mistletoe lectin can be

Table 5 Examples of lectins with antifungal activity

Natural source of lectin	Fungal species inhibited	Sugar specificity	Reference
<i>Amaranthus viridis</i> (Green Amaranth) seeds	<i>Botrytis cinerea</i> , <i>Fusarium oxysporum</i>	Asialofetuin, fetuin, T-antigen, N-acetyl-D-lactosamine, N-acetyl-D-galactosamine	Kaur et al. 2006c
<i>Astragalus mongholicus</i> (huangqi) roots	<i>Botrytis cinerea</i> , <i>Colletotrichum</i> sp., <i>Droschslara turia</i> , <i>Fusarium oxysporum</i>	D-galactose, lactose	Yan et al. 2005
<i>Capparis spinosa</i> (caper) seeds	<i>Valsa mali</i>	D(+)-galactose, α -lactose, raffinose, rhamnose, L(+)-arabinose, D(+)-glucosamine	Lam et al. 2009
<i>Capsicum frutescens</i> (red cluster pepper) seeds	<i>Aspergillus flavus</i> , <i>Fusarium moniliforme</i>	D-mannose, glucose	Ngai and Ng 2007
<i>Curcuma amarissima</i> Roscoe (wei ji ku jiang-huang) Rhizomes	<i>Colectrotrichum cassiicola</i> , <i>Exserohilum turanicum</i> , <i>Fusarium oxysporum</i>	Not found	Kheeree et al. 2010
<i>Dendrobium findlayianum</i> (orchid) pseudobulbs	<i>Alternaria alternata</i> , <i>Colletotrichum</i> sp.	Not found	Sattayasai et al. 2009
<i>Phaseolus vulgaris</i> cv “flageolet bean” seeds	<i>Mycosphaerella arachidicola</i>	Not found	Xia and Ng 2005
<i>Phaseolus vulgaris</i> cv “French bean 35” seeds	<i>Valsa mali</i>	Not found	Lam and Ng 2010b
<i>Phaseolus coccineus</i> seeds	<i>Gibberella sanbinetti</i> , <i>Helminthosporium maydis</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i>	Sialic acid	Chen et al. 2009a
<i>Phaseolus vulgaris</i> cv “red kidney bean” seeds	<i>Coprinus comatus</i> , <i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i>	Lactoferrin, ovalbumin, thyroglobulin	Ye et al. 2001
<i>Pouteria torta</i> (pouteria trees/eggfruits) seeds	<i>Saccharomyces carevisiae</i> , <i>C. musae</i> , <i>Fusarium oxysporum</i>	Fetuin, asialofetuin, heparin, orosomucoid, ovalbumin	Boleti et al. 2007
<i>Talisia esculenta</i> (pitomba) seeds	<i>Microsporium canis</i>	D-mannose	Pinheiro et al. 2009
<i>Withania somnifera</i> (Ashwagandha/ Indian ginseng/Winter cherry/ Ajagandha/Kanaje Hindi/ Amukkuram) leaves	<i>Fusarium moniliforme</i> , <i>Macrophomina phaseolina</i>	Not found	Ghosh 2009
<i>Zea mays</i> (maize) endosperm	<i>Aspergillus flavus</i>	D(+)-galactose	Baker et al. 2009

used in cancer patients to improve the quality of life (Semiglazov et al. 2006).

In order to widen the application of anti-tumor lectins, the mechanism of action was elucidated. Lectins elicit apoptosis in different cancer cell lines. Examples include Korean mistletoe lectin-treated B16-BL6 melanoma cells (Park et al. 2001), Korean mistletoe lectin-treated human A253 cancer cells (Choi et al. 2004), *Agrocybe aegerita* lectin-treated HeLa cells (Zhao et al. 2009), Abrus agglutinin-treated Dalton's lymphoma cells (Bhutia et al. 2008a) and HeLa cells (Bhutia et al. 2008b), *Sophora flavescens* lectin-treated HeLa cells (Liu et al. 2008), *Polygonatum odoratum* lectin-treated murine fibrosarcoma L929 cells (Liu et al. 2009b), *Polygonatum cyrtonea* lectin-treated human melanoma A375 cells (Liu et al. 2009a), *Pseudomonas aeruginosa* hemagglutinin-treated breast cancer cells (MDA-MB-468, and MDA-MB-231HM cells; Liu et al. 2009c), French bean hemagglutinin-treated breast cancer MCF-7 cells (Lam and Ng 2010a), and recombinant protease-resistant galectin-9-treated myeloma cells (Kobayashi et al. 2010). The apoptotic factors involved are summarized in Table 6.

Although the apoptotic pathways look different, activation of different caspases is usually involved. Caspase-3 plays a central role in apoptosis. It interacts with caspase-8 and caspase-9. Therefore, caspase-3 is usually investigated in apoptotic pathways, except in the case of a caspase-3-

deficient cell line (e.g., MCF-7 cells) which was used in the study of French bean hemagglutinin (Lam and Ng 2010a). Caspase-8 and -9 are also activated (Liu et al. 2009a; Liu et al. 2009b, c; Kobayashi et al. 2010; Lam and Ng 2010a).

Apoptosis can be mediated by death receptors initiated by lectins. FAS receptor is the receptor with which lectins often interact (Liu et al. 2009b, c; Lam and Ng 2010a). The interaction is probably by protein–protein interaction.

The Bcl family members (anti-apoptotic factors) were down-regulated (Bhutia et al. 2008a, b; Liu et al. 2009a; Lam and Ng 2010a). The sequestration of cytochrome c in mitochondria was interrupted. Cytochrome c release was observed (Bhutia et al. 2008a, b; Liu et al. 2009a, b; Lam and Ng 2010a). Finally, mitochondrial membrane depolarization was detected (Liu et al. 2009a, b; Lam and Ng 2010a).

G0/G1 arrest was frequently observed (Bhutia et al. 2008a, b; Liu et al. 2009c; Lam and Ng 2010a). It seems that it is the characteristic of lectin-induced apoptosis. Although sub G1 arrest (Park et al. 2001) and G2/M arrest (Lam and Ng 2010a) were found in some cases.

Investigations of the anti-tumor effect of lectin in vivo have been reported. *Pleurotus citrinopileatus* lectin (Li et al. 2008) and *R. lepida* lectin (Zhang et al. 2010) exerted potent antitumor activity in white Kunming mice bearing sarcoma 180, and caused inhibition of tumor growth when administered intraperitoneally.

Table 6 Apoptotic modulation of lectin-treated cancer cells

	FAS L	Caspase-9	Caspase-8	Caspase-3	Cytochrome c	Bcl family	DNA fragmentation	Morphological changes	Sub G1	G0/G1	Mitochondrial transmembrane depolarization
1 ^a								+		+	
2 ^b				+							
3 ^c				+	+	+	+			+	
4 ^d				+	+	+	+			+	
5 ^e			+	+			+				
6 ^f	+	+	+	+	+						+
7 ^g		+	+	+	+	+					+
8 ^h	+	+	+	+						+	
9 ⁱ	+	+	+		+	+		+		+	+
10 ^j		+	+	+							

+ Parameter affected by lectin

^a Korean mistletoe lectin-treated B16-BL6 melanoma cells (Park et al. 2001)

^b Korean mistletoe lectin-treated human A253 cancer cells (Choi et al. 2004)

^c Abrus agglutinin-treated Dalton's lymphoma cells (Bhutia et al. 2008a)

^d Abrus agglutinin-treated HeLa cells (Bhutia et al. 2008b)

^e *Sophora flavescens* lectin-treated HeLa cells (Liu et al. 2008)

^f *Polygonatum odoratum*-lectin treated murine fibrosarcoma L929 cells (Liu et al. 2009b)

^g *Polygonatum cyrtonea*-lectin treated human melanoma A375 cells (Liu et al. 2009a)

^h *P. aeruginosa* hemagglutinin-treated breast cancer cells (MDA-MB-468, and MDA-MB-231HM cells) (Liu et al. 2009c)

ⁱ French bean hemagglutinin-treated breast cancer MCF-7 cells (Lam and Ng 2010a)

^j Recombinant protease-resistant galectin-9-treated myeloma cells (Kobayashi et al. 2010)

The aqueous extract of European mistletoe (*Viscum album*, L.) has been applied in cancer therapy (Lyu et al. 2004). However, in order to make lectin useful practically in the clinical setting, a delivery system is required to lower toxicity, extend exposition, and improve efficacy. Wheat germ agglutinin and *Ulex europaeus* agglutinin displayed strong interaction with human urinary carcinoma 5,637 cells, which enabled them to target to bladder cancer cells (Plattner et al. 2008). The encapsulation of *Cratylia mollis* lectin with liposomes lowered its tissue toxicity in the liver and kidney, and improved its antitumor activity in Swiss mice inoculated with sarcoma 180 (Andrade et al. 2004). Mistletoe lectin was stabilized with alginate/chitosan microcapsules coated by a biodegradable polymer wall which can be used to protect the lectin from acidic pH in the stomach (Lyu et al. 2004).

Immunofluorescence and/or immunohistochemical studies using lectins can reveal the early premalignant stage of prostate carcinogenesis. Expression of glycoconjugates is often altered in tumor cells. Abundant N-acetylglucosamine (α 1,3) N-acetylglucosamine/galactose and galactose (β 1,4) N-acetylglucosamine (α ,2) mannose (α 1,6) residues were observed in dysplastic epithelium tumor cells as evidenced by labeling by the N-acetylgalactosamine-specific and complex-type oligosaccharide-specific lectins. The binding of these lectins to androgen-independent noble rat prostatic carcinoma was revealed, indicating that these sugar residues are common in some dysplastic and neoplastic prostatic cells (Chan et al. 2001).

Lectins-antiviral drug

The D-mannose-specific lectin from *Gerardia savaglia* was firstly reported to prevent infection of H9 cells with human immunodeficiency virus (HIV)-1. Furthermore, the lectin inhibited syncytium formation in the HTLV-III_B/H9-Jurkat cell system and HIV-1/human lymphocyte system by reacting with the oligosaccharide side chains of the HIV-1 gp120 envelop molecule (high-mannose oligosaccharides; Müller et al. 1988). A year later, the lectins concanavalin A, wheat germ agglutinin, *Lens culinaris* agglutinin, *Vicia faba* agglutinin, *Pisum sativum* agglutinin and phytohaem (erythro)agglutinin were found to bind to gp120. They were able to inhibit fusion of HIV-infected cells with CD4 cells by a carbohydrate-specific interaction with the HIV-infected cells (Hansen et al. 1989). Plant lectins displayed anti-coronaviral activity, especially mannose-binding lectins, in severe acute respiratory syndrome coronavirus. They interfered viral attachment in early stage of replication cycle and suppressed the growth by interacting at the end of the infectious virus cycle (Keyaerts et al. 2007). Banana (*Musa acuminata*) lectin inhibited HIV replication (Swanson et al. 2010).

The treatment of AIDS with lectins was further investigated. Different lectins have different anti-HIV mechanisms. More recently, lectin from the polychaete marine

worm *Chaetopterus variopedatus* inhibited cytopathic effect induced by HIV-1 and the production of viral p24 antigen (Wang et al. 2005). The sea worm (*Serpula vermicularis*) lectin suppressed the production of viral p24 antigen and cytopathic effect induced by HIV-1 (Molchanova et al. 2007). *P. cyrtoneuma Hua* lectin inhibited HIV-I- and HIV-II-induced cytopathicity in MT-4 and CEM cells (An et al. 2006). Banana lectin directly bound the HIV-1 envelope protein (gp120) and blocked entry of the virus into the cell, and decreased the levels of the strong-stop product of early reverse transcription (Swanson et al. 2010). Extralong autumn purple bean lectin (Fang et al. 2010) and mushroom *Russula delica* lectin (Zhao et al. 2009) were able to inhibit HIV-1 reverse transcriptase. Hence, lectins are potential drugs for treatment of AIDS.

Besides the aforementioned practical applications of lectins, there have been isolated reports of the antibacterial (Ngai and Ng 2007) and anti-nematode (Wang et al. 2005) activities of lectin.

Other applications

Lectin affinity chromatography is a form of chromatography in which lectins are immobilized on gel beads. Glycoproteins are then adsorbed by lectins and eluted with a specific carbohydrate. So, it can be employed to fractionate and purify glycoproteins based on their specific features. Moreover, serial lectin column chromatography is useful for isolation of extremely small amounts of glycoproteins. In combination with other separation techniques, oligosaccharides can be purified rapidly.

Eight lectin-Sepharose columns were used to separate different commercial types of recombinant human erythropoietin, erythropoietin analogues and urinary human erythropoietin from healthy individuals (Franco Fraguas et al. 2008). Lectin affinity chromatography, such as immobilized Concanavalin A, was applied in the isolation of glycopeptides that express biantennary and hybrid N-linked structures and high-mannose glycans, which are abundant in both embryonic stem cells and embryo bodies stages (Alvarez-Manilla et al. 2010).

The structures of glycoproteins are difficult to analyze because of the linked carbohydrates. Lectin microarray provides a solution. Panels of lectins are immobilized on a single chip in an array format. The fluorescent-tagged samples are hybridized to the array. After analysis of the spots-binding pattern, information on carbohydrate composition of the sample can be obtained, even if the glycosylation of samples is small (Pilobello and Mahal 2007). Most recently, lectin microarray was applied in distinguishing mammalian cells that were infected with the intracellular apicomplexan parasite *Cryptosporidium parvum*. The cells were probed with fluorescent-labeled lectins. N-acetyl-D-galactosamine binding soybean agglutinin generated the largest signal difference due

to the over-expression of glycoprotein on the surface of infected cells and the glycoprotein located in the intracellular parasites (Yang et al. 2010).

The carbohydrate specificity of lectins is exploited to recognize samples. The steroid hapten digoxigenin-conjugated lectins enable immunological detection of the structures of the bound lectins. Lectins specifically identifying the terminal sugars are used, thus allowing the carbohydrate chain to be identified. It was used to modify the glycosylation patterns of cell surface glycoconjugates during thymocyte selection processes during postnatal period in mice (Balcan et al. 2008).

Lectins from seeds of *Vicia cracca* react specifically with human blood group A erythrocytes (Rüdiger 1977). A handful of lectins may be considered as excellent reagents for anti-A, anti-B, anti-N etc., but the anti-A and anti-M antisera are not yet regarded as commercially suitable. Lectin from *Dolichus biflorus* can be used as anti-A, and lectin from *Griffonia simplicifolia* as anti-B. Lectin from *Vicia graminea* is said to be a good typing reagent as anti-N (Khan et al. 2002).

Outlook and perspectives

Lectins are produced by a myriad of organisms, some of which are phylogenetically remote. Hence, it is not surprising to find that mushroom lectins are very different from plant lectins and animal lectins in amino acid sequence, molecular weight, number of subunits, sugar specificity, thermostability, and pH stability. Isolectins are produced by some mushrooms, e.g., *Tricholoma mongolicum* (Wang et al. 1995), some plants, e.g., *Narcissus tazetta* lectin (Ooi et al. 1998), and some animals, e.g., *Conger myriaster* (Muramoto et al. 1999). Despite disparity in physicochemical and biochemical characteristics, lectins from different sources essentially exhibit common biological activities that encompass antitumor, immunomodulatory, anti HIV-1 reverse transcriptase inhibitory activities which can be regarded as anti-pathogenic activities. It is likely that different lectins have common intracellular effectors; it is noteworthy that the peptide hormone glucagon and the catecholamine hormone epinephrine are structurally disparate and have distinct receptors on the hepatocyte membrane and also on the adipocyte membrane. They both stimulate membrane-bound adenylate cyclase and raise the intracellular cyclic AMP level to bring about glycogenolysis (Hadley and Levine 2007). A common signaling pathway may be involved for different lectins. Sometimes different pathways are affected, for instance, in their apoptosis-inducing action (Table 6). Of course, it is reasonable that different signaling pathways are employed for different actions, e.g., antiviral and antitumor actions of a lectin. Just like insulin, the ras-dependent pathway ensues in protein synthesis and cell division. On the other

hand, the ras-independent pathway brings about glucose uptake and glycogen synthesis (Hadley and Levine 2007).

Lectins are a subject of intense investigations. As more lectins are isolated and further studies are conducted on the biological activities and mechanisms of action of lectins, the production of lectins can be improved and new applications of lectins can be found.

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