The areas of application for plant lectins

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Lectins, in particular from plants, are proteins of non-immune origin that are able to bind carbohydrates with high specificity. Due to their properties, phytolectins are of great interest in practical applications. They were shown to play an important role in forming strategies for treatment of disease including cancer and HIV. Plant lectins are an important tool in glycomic studies. Plant lectins with fungicidal and insecticidal activities are used in transgenic technologies to increase plant resistance to pests and phytopathogens. The introduction of lectin-like kinases genes into plant genome was shown to be a perspective way to protect plants against environmental stresses and regulate plant growth. Engineering of phytolectins allows obtaining molecules with known carbohydrate specificity that can be applied in various areas. The studies are underway with the aim of design of lectin-based drug delivery systems as well as the pharmaceutical drugs containing plant lectins. Because of the ability of phytolectins to bind to different substances they can be more widely used in the future. The review focuses on current data and future possibilities in the application of plant lectins.

Keywords: plant lectins, biotechnology, glycome analysis, biomedical research, agriculture.

Introduction. Lectins are a class of proteins which selectively and reversibly bind to certain carbohydrates or carbohydrate ligands of complex biomolecules. Most lectins can bind to red blood cells [1]. This makes possible to call them «hemagglutinating proteins». Lectins have been found in plants, animals, microorganisms and viruses. Nevertheless, plants are the most accessible source of carbohydrate-binding proteins [2]. Lectins that bind carbohydrates with high specificity can be considered as decoders for information encoded in carbohydrate-containing biomolecules (glycoconjugates). Therefore lectins that decipher the glycocode are involved in various physiological processes of organisms [3]. The practical applications of plant carbohydrate-binding proteins and the development of lectin-based advanced biotechnologies are due to availability of these proteins and largely depend on their properties.

The most important characteristic of lectins is the ability to bind selectively to carbohydrates. The potential for the practical applications of phytolectins that bind to a wide variety of carbohydrates and recognize the complex glycans is significantly greater than that of other lectins. Some studies have shown that in addition to the amino acid residues forming the monosaccharide binding sites lectins can have the additional sites to bind complex molecules [4]. The multivalent interactions of plant lectins and carbohydrates [5] are of great importance in the function of these proteins. Other important property which is required for practical applications of carbohydrate-binding proteins is their stability at high temperatures [6] or low pH [7]. The resistance of lectins to digestive enzymes allows using these proteins in protection of plants against insects and in oral pharmaceutical preparations [8].

To use lectins for treating diseases it is necessary to consider their potential effects on the blood since these proteins are able to agglutinate erythrocytes. It has been shown that lentil lectin and concanavalin A increased the osmofragility of rabbit red blood cells [9].

The plant lectins that are very similar to each other can exhibit the differences in their biological activities [10]. So, the minor differences in lectin binding to cer-
tain sugars have led to some changes in the formation of legume-rhizobia symbiosis [11]. Therefore it is necessary to study the properties of each lectin in details before using this protein in biotechnology.

**Modified lectins.** The significance of lectins as substances with biological activity shows the need for engineering these proteins, mainly their carbohydrate binding domains. Such approach for obtaining the lectins of known carbohydrate specificity offers new possibilities in the practical applications of carbohydrate-binding proteins. Modified lectins can be applied for glycose analysis, diseases diagnosis or therapy.

**Mutated plant lectins.** Site-directed mutagenesis in the carbohydrate-binding domains of plant lectins, which leads to proteins with known carbohydrate specificity, is one of the ways to modify lectins [12]. So, the mutated Maackia amurensis hemagglutinins were found to distinguish putative glycoforms of immunoglobulin A1 from immunoglobulin A nephropathy patients [13]. Besides, new mutated plant lectins can be useful for the highly specific identification of different types of cells [14].

**Chimeric lectins.** Another approach for obtaining the lectins with new carbohydrate-binding properties is the construction of chimeric lectin molecules by replacing some amino acids residues with other residues [15]. For example, the treatment of bacteria from nodules of Astragalus cicer with hybrid lectin PSL/AGL (bind galactose and mannose) constructed on the basis of pea lectin (bind mannose) with the carbohydrate binding region of A. glycyphylllos lectin substituted for the corresponding PSL region led to the formation of nodules by microorganisms on alfalfa roots in contrast to control. This suggests that effect of hybrid lectin is due to broadening the range of carbohydrate ligands binding to this protein [16].

**Fusion proteins containing plant lectins.** Fusion proteins are shown to have a significant synergistic effect as biologically active compounds due to combining beneficial properties from all components of which they consist. It was shown that the scN-rGSII fusion protein composed of soyacystatin N, a soybean cysteine protease inhibitor, and lectin from Griffonia simplicifolia (rGSII) inhibited the development of cowpea bruchid (Callosobruchus maculatus) to a greater extent than the mixture of individual proteins [17]. The association of two lectins, Allium sativum lectin (ASAL) and A. cepa agglutinin (ACA) has led to fusion lectin that revealed strong inhibitory effect on the development of larvae Lipaphis erysimi, a pest of Brassica juncea. It is important that the toxicity of complex lectin against insect was observed in artificial feeding and transgenic plants [18]. Plant lectins being a part of the fusion proteins with anti-insect activity can also act as a carrier protein to deliver toxic substance to insect haemolymph [19].

**Lectin-based delivery systems.** Taking into consideration the properties of phytolectins, mainly their carbohydrate-binding ability, the resistance to digestive enzymes as well as the capacity of these proteins to mediate cytoadhesion, they are of great importance in the development of lectin-based delivery systems. Targeted delivery of drug promotes bioavailability of biologically active substances [20, 21] that is especially important for poorly absorbed compounds. Besides, targeted drug delivery to selected sites allows reducing drug toxicity and interchangeable target potential [21]. For instance, wheat germ agglutinin (WGA) was shown to have potential as a carrier for drug delivery [22].

There are two approaches to the formulation of lectin-based drug delivery. In the first case lectins play a role of the glycotargeting moiety while the drug is an active component. This construction represents prodrug. The second approach is used to create the lectin-grafted carrier systems [20], in which lectins deliver the microparticles, nanoparticles or liposomes containing the drug to the targeting site.

**Applications of phytolectins for plant protection.** The search for substances of biological origin to protect plants from the damages caused by pests and environmental stresses has been actively carried out for recent years. Some plant lectins are shown to possess insecticidal and fungicidal activities. For example, Glechoma hederacea lectin inhibited the development of Leptinotarsa decemlineata larvae [23] whereas WGA revealed a significant antibiotic effect on growth of pathogenic fungal species of Fusarium [24]. Plant lectin receptors kinases can play a significant role during seed germination as well as in plant responses to salt and osmotic stresses [25] and in the suppression of the insect-mediated inhibition induced defense responses of plants [26]. Jacalin-related lectins also have a great significance as plant defense proteins [27] and for plant growth [28].
Transgenic plants with introduced lectin genes. Lectins with anti-pest and anti-phytopathogen activities were shown to be perspective for plant protection when lectin genes were introduced into plant genome [29, 30]. So, stinging nettle agglutinin reveals antifungal activity in transgenic tobacco [29]. The survival and fecundity of the tobacco aphid, Myzus nicotianae were reduced when the gene encoding Zephyranthes grandi-flora agglutinin was integrated into the genome of Nicotiana tabacum [30]. The transgenic plants expressing, for example, bean α-amylase inhibitor 1, a lectin-like protein from bean seeds were also resistant to pests [31]. Some phytolectins can confer resistance to transgenic plants against nematodes [32]. The use of lectins for plant transformation can be considered as a way to protect plant from virus infection including the viruses that are transmitted by insects after eating plants. It was shown that the plants carrying the gene of lectin with an anti-insect activity had low virus levels [33]. A putative mechanism of action of lectins expressed in transgenic plants is to antagonize plant feeding insects by suppressing animal nutrient supply [34]. On the other hand, phytolectins are able to bind to sites for virus in insect gut and block them [35]. Along with systemic expression of anti-insect lectin the tissue-specific one was found to be very prospective approach in plant protection [36]. In spite of great importance of lectins in plant protection they can be toxic to humans and animals [37]. This should be taken into consideration before creating transgenic plants.

Recent studies have shown new directions in genetic engineering of plants to enhance their resistance to stresses. So, the introduction of two lectin genes into plant genome [38] or lectin overexpression [39] could result in a significant enhance of plant resistance to pests and phytopathogen microorganisms. Besides, the introduction of genes encoding lectin-like receptor kinases into plant genome is a promising way to protect plants from stresses [40] and to improve plant growth and development. Taking into consideration an important role of jacalin-related lectins in plant physiological processes these proteins also are of great interest for genetic engineering to increase plant productivity.

Exogenous plant lectins. Exogenous phytolectins were also shown to protect plants against pests. For instance, the significant reduction in oviposition by cowpea seed beetle, Callosobruchus maculates has been observed as a result of treatment of Cicer arietinum seeds with plant lectins. [41]. However, the lectin inhibitory activity diminished with increasing an insect density. Some phytolectins with an antifungal activity introduced into the plant rhizosphere with plant growth promoting bacteria can reduce the number of phytopathogenic microorganisms in the root zone.

Lectins improve plant-microbe symbiosis formation. As a result of the development of symbiotic interactions between rhizobia and legumes the nodules are formed on plant roots. In these specialized organs on legume roots the atmospheric nitrogen is converted into compounds that are available for plants. Lectins were shown to be involved in interaction of legumes and nodule bacteria [42] and carbohydrate-binding sites of lectins play essential role in biological activity of these proteins [43, 44].

Genetically modified plants. Transgenic plants for lectins were designed to study the role of these proteins during the legume-rhizobia symbiosis. Nodules were formed on the roots of transgenic plants carrying the genes of some lectins whereas the introduction of genes encoding other lectins into leguminous plants has led to the development of nodule-like structures [45]. For example, the expression of pea lectin in transgenic red clover roots caused the formation of primordia similar to root nodule after the inoculation of plants with pea and alfalfa nodule bacteria, which normally do not form symbiotic relationships with red clover [44].

Effect of exogenous plant lectins. It has been shown that soybean lectin as a component of lectin-bacterial preparation was able to improve the development of legume-rhizobium symbiosis [46, 47]. In addition, soybean lectin introduced into rhizobial suspension increased the level of symbiosis nitrogen fixation [46] and improved plant productivity [48]. The effects of legume lectins on symbiosis can be related to the ability of these proteins to induce the metabolic changes in bacterial cells and adhesion of microorganisms to root surface [43].

Legume lectins can be used as a biotechnological approach to improve the growth of microorganisms in vitro [49] as well as to induce the bacteria biofilm formation in the absence of the host plants (in the off-season) [6] and support the microbial activity in rhizo-
sphere. However, some agrochemicals accumulated in soil can prevent the development of lectin-mediated plant-microbe [50] or microbe-microbe interactions because of their ability to bind phytolectins.

**Lectins for plant growth improvement.** It was shown that the application of plant lectins to crop seeds improved seed germination [51] and plant growth [52]. On the other hand, lectins can improve plant growth indirectly by means of increasing biological activity of rhizosphere microorganisms. So, potato lectin introduced into the suspension of bacteria of the genus *Azotobacter* enhanced the plant growth promoting potential of microorganisms resulting in increasing potato production [53]. Plant lectins were shown to be able to stimulate nitrogen fixation in rhizobacteria [54]. The nitrogen compounds accumulated in root zone due to microbial nitrogen fixation can be utilized by plants for growth and development.

At the same time phytolectins can be used to protect rhizobacteria against adverse environmental effects, in particular heavy metals [55]. It is especially important in current environmental situation in the world. It was shown that the introduction of legume lectin gene encoding pea lectin into rice plants led to colonization of non-legume root epidermal cells with nodule bacteria [56]. Taking into consideration the fact that nodule bacteria are able to synthesize plant growth promoting substances [57], the establishment of transgenic plants carrying lectin genes to improve monocot plants growth seems to be a promising way.

**Phytolectins in glycan analysis.** Lectins are a valuable tool for study of surface carbohydrate determinants of plant, animal and microorganisms cells. The glycosylation of organic molecules plays an important role in different processes associated with cells [58] and the cell glycan profile is a glycocode which provides the identification of cells and the detection of pathogenic changes in them. It was shown that plant lectins can be used to study the glycan biomarkers on the cellular surface during oncogenic processes [59] as well as for revealing stage specific surface carbohydrates during the development of microorganisms [60] to work out a strategy for the control of microbial growth. Plant lectins were used to map the expression profile of biochemically defined saccharide epitopes in squamous epithelia [61].

**Lectin affinity chromatography.** Lectin affinity chromatography is a type of affinity chromatography when lectins immobilized on supports, for example, agarose are able to bind compounds with carbohydrate moieties providing the selective separation of biomolecules. The detailed structures of carbohydrates bound by lectins can be determined using mass spectrometry. Plant lectin-based affinity chromatography was used for protein purification [62] and cancer-associated glycoproteins identification [63] as well as to assess the polysaccharides composition of municipal waste water on a membrane bioreactor [64].

**Lectin arrays.** Lectin array, in particular lectin microarray is a new method for high-throughout glycomic analysis that uses the lectins immobilized on a solid support [65]. Modern high sensitive lectin-based microarray technologies enable to identify even monovalent oligosaccharides, which normally have a low affinity for agglutinins [66]. Lectin array is a very prospective approach for revealing the disease-associated changes in the glycosilation of cell proteins [67] or, for example, for the evaluation of cell-surface microbial sugars [68].

**Lectin histochemistry and cytochemistry.** The significance of plant lectins bound with different labels (peroxidase, colloidal gold *etc*.) as cytochemical and histochemical markers to identify glycoproteins in tissues and cells is enormous. By binding to the carbohydrate residues of cell glycoconjugates the labeled phytolectins can be used for the detection of pathological states, for example, in cancer diagnosis [69] as well as in the histochemical study on the plant-microbe systems formation. So, the labeled arabinogalactane protein (β-lectin) was shown to be a probe for identification of β-related sugars in ultrathin sections of wheat leaves infected with pathogen fungus [70]. Wheat germ agglutinin conjugated with fluorescein isothiocyanate was shown to be an effective tool for the isolation and characterization of plant growth-promoting rhizobacteria [71].

The lectin enzyme-linked immuno sorbent assay [72] and lectin blot [73] can also be used for the identification of protein with changes in glycosylation.

It was shown that the combination of different methodological approaches provided a significant improvement in glycomic analysis. For example, electrophoretic protein separation and lectin array-based glycan
profiling combined into a single experiment allow developing an effective lectin array blotting technology [74].

Over the last few years a number of new techniques involving plant lectins to study cell glycans composition have been developed. One of them is the nanotechnology-based system that was set up for the high sensitive in situ identification of carbohydrates on living cells [75].

Plant lectins in medicine. Biomedical research. Plant lectins were shown to have great potential in biomedical researches. For example, the specific trans-synaptic neural pathway was visualized by the introduction of wheat germ agglutinin cDNA as a transgene [76]. Besides lectins, which have an appreciable affinity for sites of human immunodeficiency virus 1 (HIV-1) responsible for infection of human cells is a useful tool for studies of causes of HIV infection [77] in order to find the ways by which this disease can be prevented. Phytolectins are of great importance to investigate changes in cell glycosilation for tumor biomarkers [73] and to determine tumour marker glycoproteins [78] or to study human erythrocyte membranes, for example, in the case of permanent radiation [79]. The legume lectins with different carbohydrate-binding affinities were used to determine the effect of carbohydrate residues of cell membranes during inflammatory process in vivo. It is suggested that the anti-inflammatory activity of legume lectins may be due to a blockade of neutrophil-selective carbohydrate ligands, the major ligand on rat neutrophil of which is N-acetyl-glucosamine [80].

On the contrary, Arum maculatum agglutinin revealed the pro-inflammatory activity inducing neutrophil migration [81]. Carbohydrate-binding proteins can be involved in determination of enzyme activities in tissues extracts and cell cultures since they are able to bind to carbohydrate products of enzymic cleavage [82]. Plant lectins were shown to be biomarkers for monitoring pluripotency in stem cell populations [83].

Disease treatment. Recent studies showed that plant lectins can be used in medicine to treat a number of diseases. So, the lectins from the Amaryllidaceae family can be considered as a type of microbicidal proteins for prevention of human immunodeficiency virus infection [84]. The lectins from Hippeastrum hybrid and Galanthus nivalis are found to inhibit both HIV-1 and HIV-2 infections by interruption of virus entry into cells. It was shown that banana lectin inhibited the activity of HIV-1 reverse transcriptase and the proliferation of leukemia cells (L1210) [85]. Mistletoe lectins also had cytoxic effect on tumor cells [86]. The mistletoe lectin and mistletoe extract preparation standardized for mistletoe lectin-1 induced cytokine production by monocytes, which play an important role in cancer growth inhibition [87]. Sequential sustained-release preparation containing cytotoxic lectin ricin and cobra venom cytotoxin was showed to inhibit the hepatocellular carcinoma growth in vivo [88]. The inhibition of initial adhesion and biofilm formation by Streptococcus mutans resulting from their treatment by lectins with specificity for glucose and mannose revealed a new strategy in the treatment of dental caries [89]. The antidepressant-like effect of lectin from Canavalia brasiliensis seeds shown in the forced swimming test, was related to its interaction with serotonergic, noradrenergic and dopaminergic systems [90].

Conclusion. Plant lectins have been exploring for many years, however some issues remain poorly studied. Taking into consideration the ability of lectins to bind selectively to carbohydrates and considering these proteins as a type of signaling molecules triggering a variety of physiological processes in living organisms, it is possible to suggest that they have significant potential for widespread practical use. In the Figure the main actual and potential areas of plant lectin application are summarized.

There are several approaches for application of plant lectins: (i) non-immobilized lectins can be used to induce the metabolic changes in cells and tissues (immune system modulation, treatment of diseases, plant protection, microorganisms activation, plant growth regulation), to block receptors (plant protection, treatment of diseases), and to bind the cells (biofilm and plant-microbe system formation); in addition, phytolectins can be used for enzyme protection and activation, and retention of biomolecules; (ii) labeled lectins serve as probes (glycome analysis, disease diagnostics, microorganisms isolation) or lectins are used as drug carriers; (iii) immobilized lectins (lectin array, lectin chromatography) are used in glycome analysis and, for instance, diseases diagnostics; (iv) lectin genes introduced into plant genome can provide protection of plants
The actual and potential* areas of application of plant lectins

The actual and potential* areas of application of plant lectins from biotic and abiotic stresses as well as regulate plant growth and plant-microbe systems formation.

The most important area of application for phytolectins in the future is medicine. The availability of these proteins makes them a very valuable tool in disease diagnostics and therapy. The development of biotechnology involving lectins is of great importance to enhance the plant productivity because of growing demand for food in the world. Genetic engineering of plants by introduction of lectin genes is a prospective way to increase plant productivity in the future if dispute over the use of transgenic plants in agriculture is resolved in favor of the last. Increasing the activity of soil microorganisms by lectin with a view to harvest high crops [46] can be another important area of lectin application, namely development of lectin-bacterial preparations for farming. Recent studies have revealed that phytolectins are able to increase the activity of enzymes [91] as well as to stabilize enzymes against heat inactivation [92]. Therefore, plant lectins can be involved in the formation of enzyme systems for some industries. Obtaining new lectins with a wide range of useful properties from plant source [93] and using mutagenesis [15] as well as the production of recombinant lectins [94] are of great importance for the biotechnology development. The ability of lectins to bind hormones and other substances can greatly extend the fields of application of these proteins in the future.

Thus, phytolectins have a significant potential for practical applications that are closely related to the structure of these molecules and their physical and chemical properties.
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Область застосування рослинних лектинів

Резюме

Лектини, зокрема, лектини рослин є бікмами неімунного процесу, які передбачають узагальнення з високим ефективністю. Завдяки своїм властивостям фітополізми викликають значний інтерес щодо їхнього практичного застосування. Показано, що вони відрізняють важливу роль при розробці стратегії лікування захворювань, включаючи рак і ВІЛ, а також у дослідженнях гілоку-му клеток. Рослинні лектини з функціональною та інсектицидною активністю використовують у трансплантаційних технологіях для підвищення стійкості рослин до фітопатогенів і холоз. Інтродукція генів лектинових кінз у геном рослин може бути перспективним напрямом захисту від дії стресових чинників навколишнього середовища і регулювання їх росту. Конструювання фітополізми дозволяє отримувати молекули з високою багатованіс- ною специфічністю, що розширює сферу застосування зазначе- них білків. Проводять також дослідження для створення фарма- цетичних препаратів, які містять лектини рослин, та систем спрямованої доставки змій на основі лектинів. Через здатність фітополізмів взаємодати з різними речовинами вони можуть мати ширше використання в майбутньому. Одяг іхнюю сус- часні дати та перспективні напрямки практичного застосування рослинних лектинів.

Ключові слова: лектини рослин, біотехнології, дослідження гілоку, біомедічні дослідження, сільське господарство.

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Область призначення растительних лектинів

Резюме

Лектини, в частності, лектини растінів відомі як білки не- імунного процесу, за рахунок своїх властивостей з високою специфічністю. Благодаря своїм можливостям фітополіз- ми висвітлюють значний практичний інтерес. Показано, що вони играють важливу роль в розробці стратегії лікування захворювань, а також у дослідженнях гілокому клеток. Лектини з функціональною та інсектицидною активністю використовують в трансплантаційних технологіях для підвищення устойчи- вості растіння до фітопатогенів та насекомих. Інтродукція геном растенихних кінз у геном ростення може мати ширше використання в майбутньому. Конструювання фітополізмів дозволяє отримувати молекули з високою багатованіс- ною специфічністю, що розширює сферу застосування зазначе- них білків.

Ключові слова: лектини рослин, біотехнології, дослідження гілоку, біомедічні дослідження, сільське господарство.

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Received 25.12.12