Chapter 23

Biological Applications of Plants and Algae Lectins: An Overview

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1. Introduction

More than 120 years ago, Peter Hermann Stillmark in his doctoral thesis presented in 1888 to the University of Dorpat, gave the earliest step in the study of proteins that have a very interesting feature: the ability to agglutinate erythrocytes. These proteins were initially referred as to hemagglutinins or phytoagglutinins, since they were originally isolated from extracts of plants [1]. The first hemagglutinin isolated by Stillmark was extracted from seeds of the castor tree (*Riccinus communis*) and was named ricin [2]. This hemagglutinin was strongly used by Paul Ehrlich as model antigens for immunological studies [2,3].

Thirty-one years after Stillmark, James B. Sumner, isolated from jack bean (*Canavalia ensiformis*) a protein that he called concanavalin A (ConA). For the first time a pure hemagglutinin had been obtained [4]. However, the report that ConA agglutinates cells such as erythrocytes and yeasts and also precipitates glycogen from solution was given by Summer and Howell nearly two decades after its isolation. In addition, the findings of these researchers showed that the hemagglutination by ConA was inhibited by sucrose, demonstrating for the first time the sugar specificity of lectins. Thus, they suggested that the hemagglutination induced by ConA might be due to the reaction of the plant protein with carbohydrates expressed on the surface of the erythrocytes [1,4].

In 1907, Landsteiner and Raubitschek analyzed the hemagglutination of red blood cells from different animals by various seeds extracts. They found that the relative hemagglutinating activity was quite different for each extract tested [1]. However, it was only in the 1940s that Willian Boyd and Karl Renkonen, working independently, made the important discovery of human blood groups specificity for hemagglutinins. They found that crude extracts of two leguminous plants, *Phaseolus limensis* and *Vicia cracca*, agglutinated blood type A erythrocytes but not blood type B or O cells, whereas the extract of *Lotus tetragonolobus* agglutinated only blood type O erythrocytes [1,5].

The specific interaction between lectins and carbohydrates of erythrocytes played a crucial role in the investigations of the antigens associated with the ABO blood group system. In the subsequent decade, Morgan and Watkins found that the agglutination of type A erythrocytes by extracts of *Phaseolus limensis* was best inhibited by α-linked N-acetyl-D-galactosamine, while the agglutination of O cells by the extract of *L. tetragonolobus* was best inhibited by α-linked L-fucose [6].

Around thirty years after Boyd, the research on lectins reached the molecular level studies. It was clear the need to a better understanding on the structural aspects of lectins. Then, in 1972 Edelman and colleagues established the primary sequence of ConA [6]. In the same year, Edelman's group and independently Karl Hardman with Clinton Ainsworth, solved the 3D structure of ConA by X-ray crystallography [7,8].

2. What exactly is a lectin?

In 1954 Boyd and Shapleigh proposed the term lectin, from the Latin verb *legere* (which means "to select"). This term was based on the fact that these proteins have the ability to distinguish between erythrocytes of different blood types [9].

Lectins were early defined as carbohydrate-binding proteins of nonimmune origin that agglutinate cells or as carbohydrate-binding proteins other than antibodies or enzymes. However, these definitions were updated, since some plant enzymes are fusion proteins composed of a carbohydrate-binding and a catalytic domain, for instance, type 2 RIPs, such as ricin and abrin, are fusion products of a catalytically active A-chain (which has the N-glycosidase activity) and a carbohydrate-binding B-chain, both linked by a disulfide bond [10]. Furthermore, there is in nature carbohydrate-binding proteins possessing only one binding site and, therefore, are not capable of precipitating glycoconjugates or agglutinating cells [11].

Thus, in 1995 Peumans and Van Damme proposed the most suitable definition for lectins. According to the "new" definition, all plant proteins that possess at least one noncatalytic domain that binds reversibly to a specific mono- or oligosaccharide are considered as lectins [12,13].

2.1. Plant lectins

Lectins are proteins widely distributed in nature such in microorganisms, plants, animals and humans, acting as mediators of a wide range of biological events that involve the crucial step of protein-carbohydrate recognition, such as cell communication, host defense, fertilization, cell development, parasitic infection, tumor metastasis, inflammation, etc [14-15].

Peumans and Van Damme classified the plant lectins according to their overall structure. *Merolectins* consist exclusively of a single carbohydrate-binding domain (e.g. hevein, a chitin-binding latex protein isolated from the rubber tree *Hevea brasiliensis*). Since merolectins have a unique carbohydrate-binding site, they are incapable of precipitating glycoconjugates or agglutinating cells. *Hololectins* are also built of carbohydrate-binding domains. However, they contain at least two such domains that are identical or very similar. Because these lectins are di- or multivalent they can agglutinate cells and/or precipitate glycoconjugates. Most plant lectins are hololectins. *Superlectins* are built of at least two carbohydrate-binding domains. Unlike hololectins, these domains are not identical or similar. Thus, superlectins recognize structurally different sugar (e.g. TxLCI, a tulip bulb lectin that recognizes mannose and N-acetyl-galactosamine). *Chimerolectins* are fusion proteins that consist of two different chains, one of them with a remarkable catalytic activity (or another biological activity). RIPs type 2 are examples of chimerolectins [11-12].

The most thoroughly investigated lectins have been isolated from plants, particularly that extracted from members of the Leguminosae family. Legume lectins are a large group of proteins that share a high degree of structural similarity with distinct carbohydrate specificities. The subtribe Diocleinae (Leguminosae) comprises 13 genera, mostly of them from the New World. However, only 3 of these genera (i.e. *Canavalia*, *Cratylia* and *Dioclea*) are considered as the main sources for protein purification [16].

Concerning the biological activity, legume lectins are considered as enigmatic proteins. Despite the philogenetic proximity as well the high degree of similarity shared between them, they possess different biological activities such as histamine release from rat peritoneal mast cells, lymphocyte proliferation and interferon-γ production, peritoneal macrophage stimulation and inflammatory reaction as well as induction of paw edema and peritoneal cell immigration in rats [16].

2.2. Plant lectins as biotechnological tools

Significant progress has been reached in last years in understanding the crucial roles of lectins in several biological processes [17]. The importance of lectins as biotechnological tools has been established early in the studies involving its biological application. In 1960 a major step in immunology was given in order to determine the role of these proteins on the lymphocytes cell division. It was found that the lectin of the red kidney bean (*Phaseolus vulgaris*), known as phytohemagglutinin (PHA), possesses the ability to stimulate lymphocytes to undergo mitosis [18]. After these findings, many studies have been performed to evaluate the role of lectins on different models involving the immune response and its products, for instance the stimulation of citokine secretion [19], functional activation of monocytic and macrophage-like cells [20] and ROS production by spleen cells [21].

In addition to immunological studies, recent works have been investigated the influence of lectins in the field of microbiology, since lectins can be considered as valuable tools to verify the role of interaction between the pathogen and carbohydrates present in host cells and its importance to disease development. For instance, it has been proposed that the pathogen *Helicobcter pylori* infects human cells through an interaction involving a lectin [22].

3. Carbohydrates and the neoplasic process

Currently, malignancies are considered a major problem in the public health, especially given their increasing incidence and prevalence rates observed in recent decades. In this context, the malignant tumors, or cancers, account for approximately 7.8 million deaths per year, thus becoming the second greatest cause of death worldwide, only behind the cardiovascular disease [23].

The cancer can be defined as a set of more than 100 diseases that have in common the uncontrolled growth of cells, which invade tissues and organs and can spread to other body regions [23]. Thus, both the processes of cellular mutation that affects the neoplastic cell and metastasis, involve a series of genetic changes that culminate in modifications in the pattern of several receptors and signaling molecules present on the cell surface [24].

Carbohydrates are biomolecules that have enormous potential for encoding biological information. These combined-molecules (Glycoproteins and Glycolipids) are responsible for different biological interactions between the cell and the extracellular environment [26]. Regarding the neoplastic cells, the glycosylation of these proteins and lipids is changed, which generates membrane signaling molecules capable of inducing several processes directly related to tumor progression such as cell adhesion, angiogenesis, cellular mitosis and metastasis, in addition, in some cases, be responsible for inhibition of apoptosis induction triggered by the cells of the immune system [26].

Certain changes in glycans occur frequently in neoplastic cells and may be considered "tumor-specific", establishing a correlation between the stage of disease progression and prognosis of the same [25]. Some classic examples of these changes are the antigens of the ABH and Lewis system. ABH antigens are not expressed in cells of healthy human colon but significantly expressed on tumor cells [27]. Since the antigen Lewis^y can be observed in several carcinomas and has been correlated with poor prognosis in breast tumors [28]. Another example is the glycosylated antigens sialyl-Lewis^A and sialyl-Lewis^x, which are significantly up-regulated in carcinomas of the colon and appear to be related to tumor progression [29].

Thus, due to the intrinsic role of carbohydrates in the tumorigenesis, the glycosylation process as well as the identification of glycosylated antigens have been intensively focused, given the fact that glycosylation of antigens can vary extensively depending on the stage of the disease, which can provide, when properly identified, a better possibility of correct diagnosis and treatment.

4. Application of plant lectins in the diagnosis of malignant tumors

Because the peculiar characteristic of specific binding to carbohydrates, lectins have been used as tools to identify aberrant glycans expressed by neoplastic cells. Such methods have been essential to obtain a more precise diagnosis that allows a more accurate prognosis.

Several methods regarding the use of lectins as tools for recognizing aberrant glycans have been proposed in recent days [30,31,32]. The technique most common and widespread is the use of lectins in immunohistochemical assays.

In this context, the study conducted by [30], showed that leguminous lectins from *Canavalia ensiformis* (ConA) and *Ulex europaeus* (UEA-1) were used as histochemical markers of parotid gland mucoepidermoid carcinoma with low, intermediate, and high grade dysplasia. The authors stated that ConA localization in the cytoplasm and/or plasma membrane was significantly associated with neoplastic cells from the three grades of severity, whereas UEA-1 was associated with low and intermediate grade dysplasia. The authors obtained similar results after the analysis of other cell regions.

Another recent methodology was addressed by [33]. This methodology exploits the fact that glycoproteins produced by cancer cells have altered glycan structures, although the proteins themselves are common, ubiquitous, abundant, and familiar. However, as cancer tissue at the early stage probably constitutes less than 1% of the normal tissue in the relevant organ, only 1% of the relevant glycoproteins in the serum should have altered glycan structures [34]. With that in mind, the strategy to approach the detection of these low-level glycoproteins is based in: (a) a quantitative real-time PCR array for glycogenes to predict the glycan structures of secreted glycoproteins; (b) analysis by lectin microarray to select lectins that distinguish cancer-related glycan structures on secreted glycoproteins; and (c) an isotope-coded glycosylation site-specific tagging high-throughput method to identify carrier proteins with the specific lectin epitope [33].

Therefore, further analyses of lectins as biomarkers have been undertaken to improve our understanding of the processes involved in malignant tumor formation. As well as enable us to acquire new methods of identification of neoplastic cells at an early stage, enabling a better prognosis with appropriate treatment and low cost.

5. Application of plant lectins in the treatment of malignant tumors

Apoptosis is a mechanism by which cells undergo death to control cell proliferation or in response to irreparable DNA damage. It is featured by unique morphological and biochemical changes, such as nucleus condensation and margination, membrane blebbing, and internucleosomal DNA cleavage [35]. As the type I programmed cell death (PCD), apoptosis occurs through two major pathways, the extrinsic pathway triggered by the Fas death receptors, and the mitochondria-dependent pathway that brings about the release of Cytocrome *c* (Cyto *c*) and activation of the death signals under stimulus. In both ways the caspases, which belong to a family of cysteine proteases, have been well established as major players in apoptosis-causing mechanisms [36].

Several studies have demonstrated that lectins can induce apoptotic cell death. In the mitochondrial-dependent pathway, ConA treatment results in a decrease of mitochondrial membrane potential, and thus collapsing mitochondrial transmembrane potential. Cyto *c* is subsequently released, making up apoptosome with Apaf-1 and procaspase-9. After conjugating apoptosome, procaspase-3 turns into active caspase-3 that eventually triggers apoptosis [37].

In [38], it was evaluated the pro-apoptotic activity of a lectin isolated from *Artocarpus incisa* (frutalin) on HeLa cells derived from human cervical cancer. In this study, frutalin possessed a remarkable antiproliferative effect on HeLa cells. This effect was irreversible as well as time and dose dependent. When the lectins were added, serious visible cellular morphology changes were observed, possibly as a result of cell stress.

An interesting study conducted by [39] showed the pro-apoptotic caspase-dependent activity of the lectin isolated from *Astragalus membranaceus* in leukemia cell line (CML K562). These results showed a close relationship with the low expression of BCL-2 (anti-apoptotic protein) indicating that the lectin is active through the mitochondrial apoptotic pathway [40]. Furthermore, structural changes in cell membrane and different levels of Caspase-3 contributed to support their hypothesis.

Despite the apoptotic activity of several lectins have been demonstrated in different studies, the precise mechanism of how this process is triggered as well the mode of internalization is unknown until the present date.

6. Carbohydrates and the inflammatory process

The inflammation is a nonspecific event of immune response that occurs in reaction to any type of tissue injury. This process is capable of triggering a series of physiological changes such as increased blood flow, elevated cellular metabolism, vasodilation, release of soluble mediators, extravasation of fluids and cellular influx [41].

The continuity of cell recruitment and tissue damage in addition to chemical mediators released by the injured tissue as well as resident cells on site activate various mechanisms, in turn, induce the migration of more immune cells as well as increasing local tissue perfusion [42].

Both, acute and chronic inflammations have specific characteristics and the innate immune system plays a central role, since it mediates the initial response. Infiltration of innate immune cells, specifically neutrophils and macrophages, characterizes the acute inflammation, while infiltration of T lymphocytes and plasma cells are features of chronic inflammation [41,42].

As discussed previously, carbohydrates can act as the intermediates of communication in biological processes such as differentiation, proliferation and certain cell–cell interactions that are crucially important in both physiological and pathological phenomena [43,44]. The information contained in the enormous variety of oligosaccharide structures normally conjugated to proteins or lipids on cell surfaces (glycocodes) is recognized and deciphered by a specialized group of structurally diverse proteins, the lectins [44].

Galectins (formerly "S-type lectins"), an evolutionarily conserved family of endogenous animal lectins, share unique features, including their highly conserved structure, exquisite carbohydrate specificity, and ability to differentially regulate a myriad of biological responses [45].

Although galectins have been implicated in many biological activities, most of the functional studies reported to date link galectins to early developmental processes, such as neovascularization, regulation of immune cell homeostasis and inflammation [44,46,47]. Through deciphering glycan-containing information about host immune cells or microbial structures, galectins can modulate a diversity of signaling events that lead to cellular proliferation, survival, chemotaxis, trafficking, cytokine secretion and cell−cell communication [46,47].

These findings are extremely important because they demonstrate the importance of the glicocodes in the process of cell recognition and inflammation. In this context, plant lectins have been widely used to understand the pro-inflammatory mechanisms, as well as the design of new compounds with pro-healing effect.

7. Plant lectins and its immunomodulatory activity

An immune system is a system of biological structures and processes within an organism that protects against disease. In order to function properly, an immune system must detect a wide variety of agents, from viruses to cancer cells, and distinguish them from the organism's own healthy tissue [41]. The immune system is composed of many cells and molecules that act in a complex and harmonious way with the ultimate goal of annihilating the aggressive factor [42].

In the immune system, two phases of activity can be clearly established: the innate immune response and the adaptive immune response. In the innate immune response, there is the activity of cells and cytokines in a nonspecific way with the main purpose to annihilate quickly the local damaging agents. At this stage, we highlight the neutrophils, eosinophils, basophils and macrophages, cells with well-established activities but with the common function of production and release of cytokines. These cytokines are molecules with various functions in the inflammatory process, such as chemotaxis, activation of certain cell groups and increased tissue perfusion [48].

On the other hand, the adaptive immune response is composed by another set of cells that acts in a more specific way, the lymphocytes. Such cells are responsible for producing anti-bodies specific for certain invading microorganisms and the activation of mechanisms of apoptosis in abnormal cells [49].

Thus, the use of molecules capable of inducing cell recruitment as well as cytokine production and lymphocytes proliferation is of special scientific interest.

Korean mistletoe (*Viscum album* L. var. *coloratum*) is traditionally used as a sedative, analgesic, anti-spasmolytic, cardiotonic and anticancer agent, in Korea. An important lectin has been isolated from this plant and its immunomodulatory activity was analyzed [50]. It was shown that KML differentially modulated macrophage-mediated immune responses. It also enhanced the expression of various cytokines (IL-3, IL-23 and TNF-a), ROS generation, phagocytic uptake and surface levels of some glycoproteins (co-stimulatory molecules, PRRs and adhesion molecules). Nevertheless, the functional activation of adhesion molecules assessed by cell–cell or fibronectin adhesion events was up-regulated by KML treatment.

A recent study [51] demonstrated the immunomodulatory activity of ConBr, a lectin isolated from *Canavalia brasiliensis* seeds. The assays showed that ConBr was able to induce *in vitro* proliferation of splenocytes with minimal damage to the cellular structure. Furthermore, ConBr increased in the production of cytokines such as IL-2, IL-6 and IFN-γ production and decreased IL-10. These findings indicate the potential immunomodulatory effect of this lectin in conjunction with the intrinsic role of carbohydrates in intercellular communication related to the inflammatory process.

Regarding the activity of lectins on lymphocytes, a recent study [52] evaluated the effect of lectin extracted from seeds of *Cratylia mollis* Mart. (Cramoll 1,4) on experimental cultures of mice lymphocytes. In this study, aspects directly related to inflammation as cytokine production, cytotoxicity and cellular production of nitric oxide (NO) were evaluated. Cramoll 1,4 did not show cytotoxicity at the concentrations tested, in addition, was able to induce IFN-γ and showed an anti-inflammatory activity through the supression of NO production.

The biological function of carbohydrates in inflammation events is well-defined. In this context, proteins that bind specifically to such glycans are of great interest because of their possible functions and applications in biotechnological studies.

8. Plant lectins and its pro-healing activity

Recently, researches have undertaken efforts at the possible pro-healing activity of some lectins [53,54]. This goal is supported by the fact that such molecules may interfere with the inflammatory process. This effect is not yet fully elucidated, however peculiar and interesting results can be observed.

Experiment conducted in a murine model, employing the lectin isolated from *Bauhinia variegata* seeds (BVL) topically on surgically induced wounds, revealed the pro-healing potential of such molecule. Although not yet elucidated, it is suggested that this lectin appears to stimulate the mitogenic activity of resident cells, turning them into potent chemotactic agents for the recruitment of neutrophils through the release of cytokines [54]. Furthermore, it is suggested that the BVL stimulates the differentiation of fibroblasts into myofibroblast, which is an extremely important event in the remodeling of connective tissue [55].

Although promising, this issue requires further studies to better characterize the mechanisms involved in pro-healing role played by lectins.

9. Marine algae lectins

To date, there are fewer than 100 publications describing the presence of lectins in marine red, green and brown macroalgae. Moreover, and in marked contrast to higher land plant lectins, marine algal lectins have been isolated and characterized at a much lower pace since the first report of haemagglutinating activity in these organisms appeared more than 46 years ago [56]. Thereafter, other studies describing the presence and/or purification of algal lectins were reported by groups from England [57], Japan [58], Spain [59], United States [60] and Brazil [61].

Currently, the presence of lectins was analyzed at about 800 algae species. However, this number is still small, considering that there are thousands of species of marine algae. Together, the research shows that approximately 60% of the analyzed species show hemagglutinating activity. The number of positive species could be higher since in the first screenings the authors used a limited number of red blood cells and without enzyme treated erythrocytes.

The improvement in the methodologies of both, extraction and hemagglutination assays could increase the number of positive species. In fact, there appears to be coincidence that the rabbit erythrocytes treated with papain are most suited for the hemagglutinating activity detection in marine macroalgae [62,63].

Although marine algal lectins show proteinaceous content similar to lectins from terrestrial plants, they differ in some aspects. Early publications on this issue, reported that in general, lectins from algae have low molecular masses, no affinity for monosaccharides, strong specificity for complex oligosaccharides and/or glycoproteins. Moreover, they appear to have no requirement for metal ions, showing high content of acidic residues and even in high concentrations tend to stay in the monomeric form [64,65,66]. However there are a few reports showing that some of these molecules may be inhibited by simple sugars and are cation dependent as showed for the lectins from the green marine alga genus, *Codium* [67] and red marine alga genus, *Ptilota* [68,69,70].

The classical methods used to purify marine algae lectins include methods such as protein precipitation (using salt or ethanol), liquid chromatography (especially affinity) and electrophoresis [69,71]. Ion exchange chromatography has been effectively used in the isolation of lectins from seaweed, mainly in initial stages in purification. In this step, the lectins were separated from pigments present in the extracts [66,72,73]. In the protein extracts, phycobilins are co-extracted with lectins, becoming an undesirable contaminant in the purification process [72].

Lectins from marine algae *Cystoclonium purpureum* [74], *Gracilaria verrucosa* [75], *Palmaria palmata* [76], *Solieria robusta* [62], *Gracilaria tikvahiae* [60], *Bryothamnion seaforthii* and *B.triquetrum* [66], *Solieria filiformis* [77], *Enantiocladia duperreyi* [78], *Amansia multifida* [73], *Hypnea musciformis* [79], *Gracilaria ornata* [80], *Hypnea cervicornis* [81] and *Georgiela confluens* [82] were isolated by exchange chromatography, usually on DEAE cellulose. In contrast, due usually algal lectins have binding specificity of complex sugars, affinity chromatography has been used a few times, such as the lectins of green algae of the genus *Codium* [83,84,85], lectins from *Ulva lactuca* [86], *Caulerpa cupressoides* [87], *Enteromorpha prolifera* [88], *Ulva* *pertusa* [89], *Bryopsis plumosa* [90,91,92], *Bryopsis hypnoides* [93] and lectins from red marine algae of the genus *Ptilota* [68,69,70].

To date, only 31 lectins from Rhodophyceae and 17 lectins from Chlorophyceae were isolated and characterized. The virtual absence of lectins isolated from brown algae (Phaeophyta) is mainly due to the amount of polyphenols present in plants. It is known that polyphenols are released in extraction and that these compounds and their oxidation products, quinones, bind tightly to proteins [94] causing a false hemagglutination [83,95].

Even with the increase in the publications related to marine algae lectins, biochemical and structural information on algal lectins is scarce and from only a few species, and hence the functional and phylogenetic classification of these lectins remains unclear. The available structural information indicates the existence of different carbohydrate-binding proteins in the marine algae investigated. Moreover, the complete amino acid sequences of only 14 algal lectins have been determined. In red marine algae, *Bryothamnion triquetrum* lectin (BTL) was the first lectin to be determined its primary structure [96].

In same year, [97] reported the primary structure of *Hypnea japonica* agglutinin (HJA) that shares sequence similarity with BTL and with lectin from *Bryothamnion seaforthii* [98] and these lectins constitute the first marine red alga lectin family. Based on identity between HML and HCA and in the differences in amino acid sequences compared with BTL/HJA, [99] suggest that HCA/HML constitute another algal lectin family.

On the other hand, the lectins isolated from *Eucheuma serra*, *E. amakusaensis*, *E. cottonii* [100] have masses around 28 kDa, presents a monomer, share N-terminal sequence similarity with the complete amino acid sequence of isolectin 2 from *Eucheuma serra* (ESA-2) [101]. Also, the primary structure of ESA-2 contains repeated domains in their primary structure. These data suggest that lectins from genus *Eucheuma* can be grouped in a thirty family of red marine alga haemagglutinins. The amino acid sequence of lectin from red marine alga *Griffthisia sp*. [102] displays sequence similarity with lectin from jack fruit (*Artocarpus integrifolia*). The common methodology employed to determine the primary structures from red marine alga lectins was a combination of Edman degradation of sets of overlapping peptides and mass spectrometry. From green marine algae, the first primary structure determined was the lectin from *Ulva pertusa* (UPL-1) [89]. [103] described a 19 kDa protein expressed in strictly freshwater conditions in species of *Ulva limnetica Ichihara et Shimada* (freshwater alga), and this protein (named ULL) was identified and sequenced by cDNA cloning. The protein encoded by the cDNA showed 30% identity to UPL-1. However, the ULL should be considered a lectin-like since its haemaglutination activity was not yet characterized. UPL-1 and ULL did not show amino acid sequence similarity with known plant and animal lectins.

Recently [90] reported that the aggregation of cell organelles of *Bryopsis plumosa* in seawater was mediated by a lectin–carbohydrate complementary system and the purified lectin (named bryohealin) is involved in protoplast regeneration. The primary structure of bryohealin and of lectin from *Bryopsis hypnoides* [104] had few similarity with any known plant lectin, but rather resembled animal lectins with fucolectin domains. In addition, *Bryopsis plumosa*, has other two lectins described.

The lectin BPL-2 is a 17 kDa protein specific to D-mannose (ref). The authors found no similarity with others proteins in specific databases. The BPL-3 [92] possesses specificity to *N*-acetyl-D-galactosamine/*N*-acetyl-D-glucosamine and share the same sugar specificity with bryohealin. However, the primary structures of the two lectins were completely different. The homology sequence analysis of BPL-3 showed that it might belong to H lectin group from Roman snails (*Helix pomatia*). The latest primary structure published was of lectin from *Boodlea coacta* (BCA) [105]. BCA consisted of 3 internal tandem-repeated domains, each one containing the sequence motif similar to the carbohydrate-binding site of land plant lectin from *Galanthus nivalis*. It should be noted that the primary structures from green marine algal lectins were mainly determined with combination of Edman degradation and cDNA cloning.

Another observation is that a large number of sequenced algal lectins have the presence of cysteinyl residues or the duplication of internal domain. Still a lot of work needs to be done on the structure of algal lectins, since a few amount of primary structures has been determined. Further structural studies will contribute to understanding the differences in their biochemical characteristics as well as to the evolutionary aspects upon lectin presence in land plants and marine algae.

10. Marine algae lectins and its biotechnological role

There are few studies in the literature about the biotechnological applicability of lectins from marine algae. Probably due to low rentability of lectins obtained through the purification processes. It is noteworthy that the majority of lectins isolated so far were extracted from red algae, which are rich in carbohydrates and not in proteins. Moreover, during the extraction of algae proteins, phycobiliproteins are extracted simultaneously, and therefore the addition of steps to remove these phycobiliproteins causes losses of other proteins, among these are the lectins.

However, several studies on biological applications of algae lectins demonstrate that these molecules have an additional benefit; they are molecules with low molecular weight and may be less antigenic when used in biological models.

In cancerology, it was demonstrated that a lectin from the red marine algal *Eucheuma serra* (ESA) induced cell death in human cancer cells through the induction caspase-3 activity and DNA fragmentation in human colon adenocarcinoma (Colo201) cells [106]. ESA also induced cell death in a dose-dependent manner via apoptosis pathway in *in vitro* studies with Colon adenocarcinoma (Colon26) Cells derived from BALB/c mice [107].

In current studies, Span 80 vesicles (a potential type of nonionic vesicular drug delivery system) with ESA and PEGylated (EPV) lipids immobilized, showed hemagluttinating activity similar to free ESA and decreased the viability of Colo201 cancer cells *in vitro* and not affected the growth of normal cells. EPV caused the anti-tumor effect *in vivo* by inducing apoptosis in tumor cells [108].

Bryothamnion seaforthii lectin (BSL) and *Bryothamnion triquetrum* lectin (BTL) were able to differentiate human colon carcinoma cell variants. Differentiation was, probably, in function to cell membrane glycoreceptors and could be exploited to investigate structural modification of cell membrane glycoconjugates in cancer cell systems. In addition, it has been shown that the binding of both lectins to the carcinoma cells results in their internalization, which is a very interesting property that could be used in future applications, such as drug delivery [109].

The lectins isolated from the red marine algae *Hypnea cervicornis* (HCA), *Pterocladiela capillacea* (PcL) and *Caulerpa cupressoides* (CcL) have been tested as anti-inflamatory and antinociceptive agents. The data indicated that HCA, PcL and CcL have actions anti-inflammatory and antinociceptive (in formalin and acetic acid models). However, these lectins did not present significant antinociceptive effects in the hot plate test [110,111,112].

Concerning the mitogenic activity, the lectin from the red marine alga *Carpopeltis flabellate* (Carnin) was the first lectin that showed mitogenic activity for T lymphocytes from mouse spleen at a concentration of 10,5 μg/ml. Carnin inhibited the normal embryonic development of the sea urchin *Hemicentrotus pulcherrimus* at the stage of blastula and also inhibited the gasturulation induced in starfish *Asterina pectinifera,* at concentrations of 10 and 5 μg/mL, respectively. In addition, it was showed an interaction between a lectin of macroalgae with a microorganism of the marine ecosystem [113].

The antibacterial effect was too evaluated. The lectins from the red marine algal *Eucheuma serra* (ESA) and *Galaxaura marginata* (GMA) have an antibiotic activity. ESA and GMA strongly inhibited *Vibrio vulnificus*, a fish pathogen, but not were able to inactive *V. neresis* and *V. pelagius* [114]. BSL and BTL also were able to avoid the bacterial adhesion of streptococci strains in enamel pellicles. BTL was more efficient in avoiding the adherence of *Streptoccocus sobrinus* and *S. mitis* and BSL was able to reduce adherence of *S. mutans*. This fact can contribute with preventing caries at early stages [115].

Red marine algae lectins from *Amansia multifida* (AML), *Bryothamnion seaforthii* (BSL), *Bryothamnion triquetrum* (BTL) and *Gracilaria caudata* (GCL) induced neutrophil migration *in vitro* and *in vivo* in the peritoneal cavity or dorsal air pouch of rats or mice. The results showed that BT had the most potent effect in neutrophil migration when tested on rats. In mice, BS required four times higher than the dose of BT to induce neutrophil migration. However, when the algae lectins were assayed in mice, AML was the most potent [116].

Concerning the antiviral effects, a lectin named KAA-2 from the red marine algal *Kappaphycus alvarezii* (specific to high mannose type *N*-glycans) showed antiviral action against H1N1 virus [117]. The red alga lectin from *Griffithsia* sp. called griffithsin (GRFT) is a small mannose specific lectin that binds carbohydrates on HIV envelope glycoproteins and block HIV entry into target cells. GRFT is a candidate for development of anti-HIV microbicides [118,119]. GRFT was also able to inhibit the action of the pathogenic agent responsible for respiratory syndrome (SARS), the SARS coronavirus (SARS-CoV) [120].

11. Lectins as biotechnological tools to study the microbial biofilms

Biofilms are microbial complex communities established in a wide variety of surfaces and are generally associated with an extracellular matrix composed by several types of polymers [121]. This type of microbial association can develop on biotic and abiotic surfaces, including living tissues, medical devices and/or industrial water piping systems and marine environments [122,123,124].

Bacterial infections involving biofilm formation are usually chronic and often present a arduous treatment [125,126,127]. The growth and proliferation of microorganisms inside the biofilm provides reduction or prevents the penetration of various antimicrobial agents [128,129] and thus become extremely difficult or impossible to eradicate them [130,131,132]. For some antibiotics, the concentration required to eliminate the biofilm can be up to a thousand times higher than required to planktonic form of the same specie [127].

The ability of microorganisms to form pathogenic cell aggregates is a worldwide concern. In attempt to remedy this problem, pharmaceutical companies associated to research groups work avidly to the development of new options for the treatment of infections caused by bacteria organized in biofilms.

Biofilm formation is a process in which bacteria has a change in lifestyle, it goes from a state unicellular in suspension to a multicellular sessile, where the growth and cell differentiation results in structured communities. The biofilm begins with the setting free of microorganisms in a given area. The first microorganisms adhere initially by weak interactions, mainly of the van der Waals forces [133]. If the colonies are not immediately removed from the surface, they can anchor by cell adhesion molecules existing in the pili and / or flagella [134]. The first colonies facilitate the arrival of other cells through adhesion sites and begin to build the matrix that will form the biofilm. Only a few species are able to adhere to a surface *per se*. Others may anchor to the matrix or directly on existing colonies. Since the colonization has started, the biofilm grows through cell division and combination of the recruitment of other cells [135].

According to [136], the biofilm development occurs through three events. At first, there is a distribution of fixed cells in surface through cell motility. Then, occurs the proliferation of fixed cells by division, expanding to upward and sides forming agglomerated of cells, similar to the formation of colonies on agar plates [137]. Finally, clusters of cells attached to the biofilm are recruited by the action of the environment itself to the development of other biofilms, reaching a climax community [136]. These general stages provide guidance to the study of biofilms by bacteria furniture, although many details of regulation of this process may vary between species.

One of the biofilm-forming components of great importance in the maintenance of cell clusters is the matrix polymeric called EPS (*Extracellular Polymeric Substances*) [138].

Consisting of proteins, polysaccharides and environmental waste results in a solid structure highly hydrated with small channels between the microcolonies [139]. This matrix holds the biofilm together, one of those factors responsible for providing an increased resistance to antibiotics, disinfectants, and ultraviolet radiation [140]. In most biofilms, microorganisms that make up these agglomerates constitute less than 10% dry weight, while the extracellular matrix may contribute up to 90% [141].

The first step to biofilm formation, the early adhesion, is considered essential for colonization and infection by pathogenic bacteria. Macromolecules surfaces are directly involved in this stage [142]. Proteins known as adhesins are able to recognize specific polysaccharide substrate present on the surface to be colonized, for example, the presence of carbohydrates existing in the film of saliva that covers the teeth in the oral environment [143]. Glycidic epitopes present on surfaces of microorganisms (early colonizers) can also be recognized by these proteins to mediate an event known as coaggregation, which will start the formation of a multi-species community [144].

One etiological factor for the development of teeth biofilms is the adhesion of pathogenic bacteria in the dental enamel [145]. However, the microorganisms are not deposited directly on the tooth surface, but bind to a thin acellular layer composed of salivary proteins and other macromolecules that cover the tooth surfaces called acquired pellicle [146,147].

The acquired pellicle is formed by glycoproteins and carbohydrates that serve as receptors for bacteria containing proteins with glucan-binding domains [148]. Bacteria interact with the film by several specific mechanisms, including the interaction lectin-like involving the bacterial adhesins and receptors existing in the pellicle [148,149]. Next, other bacteria can adhere to the film as well as the bacteria pre-existing in the biofilm [150].

The event coaggregation is a phenomenon widely observed in diverse microbial communities [151,152,153]. Cells can interact in suspension, forming cell aggregates, as well as connect directly on biofilm in the process of formation. In the first case, plancktonic cells recognize specifically species genetically distinct creating the coaggregates. In other situations, coaggregates in the form of secondary colonizers can adhere on biofilm in development, a process known as coadhesion. Both cases have an important role in the integration and establishment of a mature biofilm [154].

Thus, carbohydrate residues have an important role in formation and maintainability of microbial biofilms. They act as mediators of the binding between bacteria and the surface that will serve as substrate for biofilm formation [155], as well as site of interaction between microorganisms to form cell aggregates [156,157]. Furthermore, through EPS matrix, maintains the biofilm attached in the surface, conferring a greater resistance to antimicrobial agents in general [158,159].

Molecules able to bind specifically and selectively to carbohydrates have a key importance in the development of research related to microbial biofilms. Thus, lectins have been shown as powerful tools for analyze the glycidic structures of those aggregates from microbial origin [160,161].

Studies of microbial biofilms through the interaction with lectins have two main objectives: visualization and characterization of polymeric matrix (EPS) formed by different species of microorganisms [162,163] and inhibition of oral biofilm formation by blocking the bacterial binding sites present in the pellicle of saliva in the form of glycoproteins and / or carbohydrates [164].

The application of lectins in the characterization of EPS is already widely exploited by many research groups. Lectin of wheat germ (WGA) was used as a marker of *Staphylococcus epidermidis* microcolonies, mainly in the study of the mechanisms involved in bacterial organization during the formation of the cell aggregates [165]. In a study published by the same group, WGA was used to quantify the production of GlcNAcβ-1,4n, a sugar component of the extracellular matrix involved in biofilm formation [166].

In 1980s was developed a system called ELLA (*Enzyme-linked Lectin Assay*) that uses enzyme-lectin conjugates to detect specific carbohydrate units on the surface of cells. This assay allows better detection and quantitation of the sugars by standard immunofluorescence with fluorescein-conjugated lectins [167]. The lectins concanavalin A (ConA) and WGA peroxidase-labeled were used to detect D-glucose or D-mannose and N-acetyl-D-glucosamine or N-acetyl-neuraminic acid, respectively, on the biofilm of several species [168].

Recent studies demonstrated the characteristics and distribution of glycoconjugates in cyanobacteria biofilm using lectins with different specificities. In this study the authors stated that the distribution of carbohydrates in the matrix is very variable. Based on lectin specificity, glycoconjugates produced by cyanobacteria biofilm contained mainly fucose, N-acetyl-glucosamine or -galactosamine and sialic acid [169].

Lectins may be a suitable antiadhesion agent for *streptococci*, since it has been reported that a lectin-dependent mechanism is involved in its adhesion [170]. However, the application of lectins as tools to interfere with biofilm formation is still poorly explored [115,171]. As shown by [172], plant lectins appear as a new strategy to reduce the development of dental caries by inhibiting the early adherence and subsequent formation of of *Streptococcus mutans* biofilm.

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