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LECTINS AND TETRAHYMENA – A REVIEW

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The unicellular ciliate Tetrahymena is a complete organism, one of the most highly developed protozoans, which has specialized organelles performing each of the functions characteristic to the cells of higher ranked animals. It is also able to produce, store, and secrete hormones of higher ranked animals and also react to them. It produces lectins that can bind them and has functions, which are influenced by exogenous lectins. The review lists the observations on the relationship between lectins and Tetrahymena and try to construe them on the basis of the data, which are at our disposal. Considering the data, lectins can be used by Tetrahymena as materials for influencing conjugation, for stimulating hormone receptors, and by this, mimic the hormonal functions. Lectins can influence phagocytosis and movement of the cells as well as the cell division. As Tetrahymena can recognize both related and hostile cells by the help of lectins and surface sugars, it could be surmised a complex predator–prey system. This could determine the survival of the population as well as the nourishment conditions. When Tetrahymena is pathogenic, it can use lectins as virulence factors.

Keywords: protozoan, lectins, lectin receptors, cell recognition, glycoconjugates

Introduction

According to Goldstein et al., a lectin is a carbohydrate-binding protein or glycoprotein of on-immune origin, which agglutinates or precipitates glycoconjugates or both [1, 2]. Lectins are components of plants and animals, which were recognized more than 100 years ago about the same time when the antibodies were recognized; however, they are in the front of biological research recently in glycomics [3, 4]. Lectins reversibly bind to specific mono- or oligosaccharides, and having this capacity, they are used for diagnosing sugars in solutions or on the cells' surface as well as in the study of blood groups and are involved in the research of cell biology, membrane structure, (innate) immunity, cancer, infections, signal transmission, and genetic engineering [5–7]. It was demonstrated that

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they have a role in cell recognition, adherence, and cell division. Lectins are able to aggregate immunoglobulins without provoking immune response [8], to inhibit fungal growth and to induce histamine release from mast cells and basophils; some lectins function as chaperones in protein folding. Having two or more binding sites for carbohydrates, they are able to connect cells with weak and temporary (reversible) interactions [9]. As the glycoconjugates are the binding sites for lectins on the cell surface, their binding to the glycoprotein part of hormone receptors can mimic hormonal effects. They are present and have roles in wide range of organisms, from bacteria to animals (including human). However, their exact biological role in natural conditions is not known till now. It is supposed that they have a role in sugar transport or carbohydrate storage, as well as in symbiotic and pathogenic interactions between microorganisms and hosts, in the adhesion to surfaces by microbes, etc.

The unicellular sweet-water ciliate Tetrahymena is a protozoon, which is at the highest level of protozoan phylogeny [10, 11]. It has separated and well-developed cell organelles for different functions, a highly developed recognition capacity and reaction to hormones of higher vertebrates as well as the ability to synthesize, store, and secrete these hormones [12]. It has signal transduction systems similar to mammals. Its insulin receptor is similar to mammalian ones [13, 14] and the glycoprotein component of the hormone receptors seems to be suitable for binding lectins also. There are many experiments and publications on the interrelationship between Tetrahymena (or other protozoa) and lectins; however, the results are not explained and compared, drawing conclusions. The purpose of this paper is to fill the gaps.

Lectins in Tetrahymena

By using antibodies to bean, pea, Lens, Datura, and snail lectins, the presence of these lectins was demonstrated in Tetrahymena [15]. The lectins were localized in the surface of the cells as well as inside them. The simple sugar-binding lectins are accumulating in the membranes and the hexosamine-binding lectins are accumulating in the cytoplasm [16]. Starvation of the cells changed the normally homogeneous settlement of lectins, causing a spotted accumulation in the cytoplasm and a patching in the plasma membrane. The snail lectin was an exception, remaining in homogeneous localization also after starvation.

A lectin reacting to an antibody against a sponge, *Geodia cydonium* lectin, was also found. This is a 36-kDa protein, which is localized in the mucocysts of Tetrahymena [17]. The lectin is discharged from the mucocysts under the effect of C2 ceramide and non-hydroxy fatty acid; however, the effect of its analogs

(hydroxy fatty acid ceramide, sphingomyelin, and sphingosine-1-phosphate) was neutral [18]. Glycoproteins secreted from Tetrahymena contain high-mannose components [19].

Selective study of lectins by synthetic neoglycoconjugates demonstrated that Tetrahymena contains beta-D-glucose and lactose-specific lectins while the presence of alpha-D-mannose, alpha-D-galactose, or alpha-L-fucose-specific lectins was not found [14]. The lectins found were associated with kinetics, and during cell division, they were localized in the fission furrow.

Binding of lectins

Specific sugars embedded in the plasma membrane of Tetrahymena are binding different lectins and they are named as lectin receptors. However, these receptors can specifically bind other materials, e.g., hormones, the glycoprotein part of them is recognized by the lectin, first of all by Con-A. The site of the binding is continuously changing parallel with the movement of the receptors inside the membrane, dependent on the cell cycle [20] or the pairing of the cells, when tipping is manifested [21]. A remodeling of the membrane glycoconjugates happens during starvation in the ciliary membrane [22].

A certain lectin, e.g., Con-A can bind different hormone receptors, such as (beta subunit of) insulin receptor, fibroblast growth factor receptors, platelet-derived growth factor receptors [23, 24]. The competing sugar, alpha-methyl mannopyranoside, decreased and imprinting (pre-treatment) with insulin increased the binding [25]. There are more binding sites for Con-A, than the hormone receptors mentioned, as at least 16 Con-A binding glycopeptides were demonstrated [26]. Glycoprotein synthesis inhibitors, swainsonine and deoxynojirimycin, increased lectin binding after 1 day; however, immediately after treatment decreased it [27]. Glycosylation inhibitors, such as glucosamine or 2-fluoro-2-deoxy-D-glucose, enhanced lectin (Con-A, Helix, Datura) binding [28]. If the membrane saccharides are modified by periodate treatment, lectin (Con-A) binding increases while insulin binding (to the same receptors) decreases, which shows that alteration of the receptor saccharide component can influence its binding capacity to different directions [29].

While insulin pre-treatment (imprinting) results in an enhanced binding of the hormone and Con-A, the lectin is provoking only a short-term imprinting for itself and it is not able to provoke this for insulin [30]. There is a similar phenomenon studying the overlap between histamine and Con-A in case of histamine receptors [31]. Histamine antagonists structurally similar to the basic molecule compete with Con-A; however, phenindamine, a structurally different

histamine antagonist does not work in Tetrahymena. In this case, the binding and effect are also separated [32]. Considering the overlap between the hormones and lectins, the hormonal influence to lectin binding was also demonstrated [33].

The culturing (environmental) conditions (temperature, osmolality, illumination, and temporary starvation) as well as the changes in the membrane fluidity basically influence the lectin-binding capacity of Tetrahymena [22, 34, 35]. Phenothiazines and local anaesthetics are also effective and characterized by the changes of lectin binding [36].

Tetrahymena secretes enzymes, if it is cultured in inorganic medium. These enzymes decrease its lectin-binding capacity [37].

Lectins can be used as anti-protozoal agents in therapy, as some of them have high cytotoxic activity, e.g., against Acanthamoeba or Tetrahymena by binding to the target cells [5].

Lectins and functions

Many life functions of Tetrahymena are influenced by lectins, as the glycoconjugates, which are components of the plasma membrane, and hormone receptors are recognized and bound by them.

Receptor-mediated "phagocytosis" [studied by the help of rhodamine (TRITC)-labeled Con-A] was increased, and mannose inhibited the binding of Con-A [38]. The increase is long lasting: the presence of it can be observed also after 40 generations [39]. Non-receptor mediated phagocytosis is also increased by Pseudomonas lectin [40].

Pseudomonas lectins (hemagglutinins) enhance the "growth" of *Tetrahymena pyriformis* and this is inhibited by specific sugars [41]. Other lectins also influenced cell division [42].

"Conjugation" of *T. pyriformis* is inhibited by Con-A [43–45]; however, it is reversed in the presence of a mannoside. Other lectins (phytohemagglutinin, soybean agglutinin, wheat germ agglutinin) do not inhibit the conjugation. During conjugation, there is a rearrangement of membrane structures, including Con-A receptors [46].

Con-A "aggregates" Tetrahymena in cultures and this effect is enhanced by imprinting (pre-treatment) with lectin or insulin. Lectins with similar sugar specificity as helix lectin have similar effect; however, lentil lectin is less active [47].

Con-A binding to insulin receptors increases the "hormone synthesis" of Tetrahymena, in general, similar to the hormone production of rat immune cells. Lower concentrations of the lectin are more effective [48].

In the study of "chemotactic effect" of lectins, the higher concentrations (above 10-9) demonstrated higher effects [49]. Considering the chemotactic selection, mannose- or galactose-specific lectins had an advantage [50].

Con-A inhibits "cell–cell recognition" in *T. thermophila* [51] and by this, inhibits conjugation [52]. The inhibitory activity of other mannose-specific lectins, as lentil and pea lectins, is tenfold lower than the effect of Con-A [53].

Con-A inhibits food vacuole "egestion" in the cytoproct of cells (only extracellular lectin has this effect), which is inhibited after digestion of Con-A [54].

"Movement" (swimming) of Tetrahymena is inhibited by Con-A and increases ciliary reversal [55]. Mannoside containing Con-A receptors is involved in the process as well as signal transduction [56].

Conclusions

The biological role of lectins is not cleared. They can be used for demonstrating glycoconjugates on the cell surface or for stimulating the mitosis of lymphocytes. However, in these cases, they are used as tools and this tells nothing on their biological role (functions). In the nature, all materials have some functions, and this is only hypothetical and speculative in the case of lectins. This is not otherwise when Tetrahymena is the object studied, so the working of fantasy is allowed.

The relationships between the protozoa and especially Tetrahymena and lectins were not studied systematically. However, the data (experiments), which are at our disposal, unanimously show that Tetrahymena synthesize, store, bind, and secrete lectins. This is not surprising, such as at the lowest level of evolution, bacteria also have these properties [57] and viruses also contain lectins (glycan binding proteins, hemagglutinins), which serve the fusion of the viral envelope with the plasma membrane of target cell and help for adhering to different surfaces [8]. This means that protozoa are not at the lowest level of evolution, where lectins are present and have a role.

In addition to the lectin production, Tetrahymena specifically binds lectins and reacts to them with changes of life-important functions. Considering this statement, the question is: what could be the role of lectins in Tetrahymena or in the relation between the Tetrahymena and their living or not living partners?

Tetrahymena is a free-living ciliated organism, which does not adhere to surfaces and freely swims in its natural or artificial milieu. This means that it does not require lectins for this aim. However, some species, e.g., *T. thermophila* are reproduced by conjugation and in this process, lectins could have a role.

Nevertheless, in this process, Con A has a negative role, which cannot be interpretable.

Mammalian macrophages and leukocytes engulf bacteria by the help of lectin (lectinophagocytosis) in the absence of opsonins [58]. The mannose receptors of macrophages [59] can recognize the carbohydrates of bacteria helping endocytosis [60]. These receptors have 8–10 lectin-like domains. There is a similar situation in the invertebrate world [61], where lectins have binding sites for different carbohydrates in the same molecule [62]. Protists also recognize, adhere [63], and phagocytize bacteria. In this case, its lectins can help to recognize bacterial glycoconjugates as well as its glycoconjugates can be recognized by bacteria. This can help the engulfment and use as nourishment of the prey, bacteria for the predator [63], Tetrahymena. It was demonstrated earlier that amoebae are using galactose-binding lectins for attaching *Legionella pneumophila* [64, 65] as well as *Acanthamoeba castellanii*, which uses mannose-binding lectin for phagocytizing yeast [66]. Marine planktonic dinoflagellates, *Oxyrrhis marina* and others, use also a mannose-binding lectin to recognize and attach phytoplanktonic preys [66]. As the variation of sugars is more numerous than that of the amino acids in proteins and the recognition of sugars and their variations by lectins has a very broad spectrum, the recognition system given by them would be the predecessor (or ancestor) of the immune system, and it is taking place already at a unicellular level. In addition, lectins have more than one binding sites specific for different carbohydrates by the same molecule and a flexibility, which allows the recognition of structurally related glycoconjugates. This could help Tetrahymena to recognize the other, hostile as well as nourishment unicellular organisms, and by this, helps the survival of the population. However, there are no data on the differential expression of lectins in the Tetrahymena plasma membrane and only scarce data are at our disposal on the inventor of Tetrahymena lectins. Considering this, the lectin dependence of an immune-preceding system is only a little bit more, than a theoretical conjecture. However, in mammals, it is also supposed that in the innate immunity, lectins have a role in the identification of microbes [2].

Although the use of lectin-carbohydrate recognition seems to be the most important function of Tetrahymena lectin, the engulfment of bacteria is not always advantageous for the cell. In other protists, bacteria sometimes can survive and reproduce in phagocytic vesicles and also can produce toxins for killing the host [67, 68] and after that for infecting the organism containing the protozoon. This is why Tetrahymena infection is very dangerous to many fish species [69]. However, not only fishes but also mammals, including human, are touched by different infections, as by a similar way, the *L. pneumophila* [70, 71] and *Salmonella enterica* [72], causing diseases, are transported. In addition, in some cases, the microbes during their stay in the phagocytic vesicles can be transformed to more pathogenic

and hypervirulent forms [73]. In this case, the packaging of bacteria by the protozoan vesicles is the Trojan Horse, which transports the disease [70]. Considering this fact, the bacterium has the advantage because of propagating itself. The role of lectins in this latter process was not studied till now; however, theoretically, lectins are needed for the recognition and adherence also in this case. Evidently, this suggested the idea that considering the recognition capacity and binding of lectin to protozoa, they can be used as therapeutic agents in case of protist (e.g., Tetrahymena)-caused diseases [5, 74, 75]. The main problem is that lectins also recognize saccharides of other cells, which also will be offended by the therapy.

As Tetrahymena can recognize microbes by the help of lectins produced by it, it is recognized by other protists, microbes, and hostile plants and animals, which also have sugar-binding capacities, as Tetrahymena has many sugar conjugates on its surface, which can be recognized by a lot of lectins. In addition, only a very low concentration of lectins is needed for the cytotoxic activity (leading to death) against Tetrahymena [3, 4] and the lectins also bind to intracellular structures [25]. By using the sugar–lectin, lectin–sugar biological network, a complex predator–prey system could be present.

Virulence is used to be mentioned in pathogenic bacteria or protozoa. These have virulence factors, which stimulate their infectivity and aggressivity. Tetrahymena itself, in general, is not pathogenic; however, there are such species between *Tetrahymena* spp., which are pathogenic, especially for fishes. Pathogenic protozoa, e.g., *Entamoeba histolytica*, uses lectins as virulence factors [75, 76], which have roles in their pathogenicity, including adherence, cytolysis, invasion, resistance to lysis, and in special cases (e.g., in *E. histolytica*), encystment. Most of the pathogenicity investigations were done in *E. histolytica*, as this is responsible for the death of about 100,000 people annually; however, there are no data on Tetrahymena. Nevertheless, there is a possibility that pathogenic Tetrahymena (in fishes) also have these virulence factors or also have the non-pathogenic species. In this case, the function of lectins in Tetrahymena would be broadened.

Tetrahymena secretes the lectins produced into the watery milieu around them. This means that around the cells, there are sugar-binding molecules in a relatively high concentration and these are able to bind hormone receptors, e.g., insulin or histamine receptors, and by this, influence functions of the cell. This case must be a competition between the hormones, which also secreted, and lectins. If the specific effect of a hormone is supposed, the effect of lectins disturbs the autocrine mechanism. In addition, lectins without the presence of the specific hormone can induce such changes in the cells or by the cells, which would be a characteristic only to the presence of the given hormone. Very scarce data are at our disposal on the regulation of hormone synthesis and secretion in Tetrahymena and we have no data at all on the regulation of lectin synthesis, storage and

secretion. The data are very scarce and sporadic also in the case of higher ranked animals, so there is no possibility to project other data to Tetrahymena. It seems not to be likely that Tetrahymena produces and secretes lectins to disturb its hormonal self-regulation. In this case, we must look after other possibilities in addition to the bacteria catching process.

Already at 1960, Nowell described the mitogenic activity of lectins [77]. Since this time, a plethora of papers justified this and lectins had been used as lymphocytic mitogens in immunological experiments. However, not only lymphocytes react to lectins with stimulated cell division but also several other cells, including Tetrahymena [42]. An extraordinarily broad spectrum of lectins has cell division influencing effects, plant lectins as well as lectins of microorganisms [78]. For example, in epithelial cells, the galactose-specific lectin of *E. histolytica* induces a MAP-kinase cascade, which promotes cell proliferation [79, 80]. *Aspergillus sparsus* lectin also has strong mitogenic potential [81]. Depending on the experimental conditions, the same lectin can be mitogenic, co-mitogenic, or anti-mitogenic [82]; however, it is not dependent on its carbohydrate specificity [83].

Tetrahymena can use the hormones produced by itself to regulate the functions of the cell population, in an extremely low concentration. However, it is not known whether lectins – also produced by Tetrahymena – can influence the cell functions of the Tetrahymena population and how it is regulated. If one can imagine that lectins promote cell division, the question is: what kind of lectins – produced by the cells – do it and how the stimulating and inhibiting lectins and actions collaborate?

Membrane-bound lectins are responsible for the removal of certain glycoproteins from the circulatory system in mammals [84]. There is a possibility that lectins, which are embedded in the ciliary membrane of Tetrahymena, have a similar role and the bound glycoconjugates are nourishing the cells. Considering that many actions of Tetrahymena are similar to that of mammalian cells [85, 86], this would not be surprising.

On the basis of the data, it seems to be unquestionable that lectins have an important role in the life of Tetrahymena, what means that the role of them can be deduced to the lowest level of eukaryotic phylogeny. However, this role is not yet exactly cleared similar to their role at the highest levels.

Conflict of Interest

The author declares no conflict of interest.

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