Current Topics in Biotechnology
Vol. 6, 2011

Original Communication

How much would the fungal lectin - antibodies interactions be selective?

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ABSTRACT

Both quantitative and qualitative estimations of the basidiomycetes *Grifola frondosa* and *Lentinus edodes* (shiitake mushroom) lectins binding to the specific and non-specific polyclonal rabbit antibodies were attempted. The *G. frondosa* lectin complexation with homological antibodies was shown to be characterized by greater binding constants as compared to non-homological antibodies. Therewith the values of changes in standard free energy ΔG^0 displaying a strength of both complexes were essentially the same. As for the two extracellular *L. edodes* lectins, *L*1 and *L*2, the anti-lectin *L*1 antibodies bound crude *L*2 with ten-fold-greater ELISA results compared to crude *L*1, but showed only limited reaction with *L*2 in the course of this lectin purification. The data obtained testify to universality of biospecific reactions "antigen-antibody" and "lectin-carbohydrate" at a molecular level, and could give new insight into the phenomenon of these biospecific interactions. Obviously, the actual specificity in the above processes of bio-recognition should be discussed in the especial case of the lectins and antibodies incorporation into a living system.

KEYWORDS: *Grifola frondosa*, *Lentinula edodes*, mushrooms lectins, antigen-antibody interaction, standard free energy, binding constant

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1. INTRODUCTION

Biological hierarchy of mutually dependent, consequent and definitely related processes caused biochemically by the specific interactions, consists an essence of the whole living systems. The ability to ordered biospecific interactions, which is based on the complementarity of bio-molecular parts and provides a bioinformation transfer in a cell, is the explicit peculiarity of just "living" molecules but not of a simple mixture of proteins, polysaccharides, etc. The extent of complementarity of given biomolecules' spatial moieties manages their affinity in a biological sense, accompanied by the expected cellular response. The factors providing such affinity are spatial topology ("geometry") of the potential bonding groups and conformational dynamics both of the receptor and ligand molecules [1]. Direct contact therewith is realized via the principal kinds of intermolecular interactions, i.e. hydrogen bonds, van der Waals forces, hydro-phobic effect [2]. Considering the standard collection of 20 amino acids and the four types of nucleotides as prime biochemical hardware in transferring information, a special kind of limited diversity inevitably occurs. Several orders of magnitude higher information potentiality inherent in the structural diversity of carbohydrates allows the latter to encode information for specific molecular recognition to provide the environmentally-mediated cellular contacts, and to manage the intended cellular responses along with the self-conserving reactions. It is apparently a function of participation in initial recognition and ordering

the living cell interactions with a permanently altered surrounding medium, that is appointed to carbohydrates involved in surface glycoconjugates [3]. The widespread concept in the scientific community today is that a glycosilation process to form glycoconjugates consists an integral part of carbohydrate metabolism in various organisms. This concept is based on contemporary glyco-biological findings, which confirm that one of the most important functions of carbohydrates in metabolism is related to bioinformation potential presented as a so-called carbohydrate code [4, 5]. The functioning of some metabolic systems, particularly those with an extracellular direction which utilizes the bioinformation potential of carbohydrates, is possible due to the realization of a biospecific carbohydrate-protein interaction. These interactions are realized mainly via lectins/ lectin-like substances capable of recognizing the specific carbohydrate determinants in the cellular structures [6].

All the aforesaid explains a growing interest in investigating the structure and functions of both the lectins and glycoreceptors. However, the item on universality of *in vivo* and *in vitro* manifestations of similar specific interactions seems to escape notice therewith. Thus, the lectins obtained from the higher basidiomycetes biomass exhibit a biological activity in respect to tumor cells of mammals and human [7, 8]. A resemble influence could be exerted by complex glycans and glycoproteins entering a composition of higher fungi cells [9]. Therewith it should be taken into account that, since fungi and mammals belong to two different natural kingdoms, so a high specificity and activity of those fungal metabolites against the animal tissues seems to be surprising and unreasonable at first glance. The new challenging researches into the properties basic for such the universal functioning of bioactive glycoconjugates would take over the task of solving a problem whether the model-simulated results could be unambiguously extrapolated to the actual living systems for the purposes of medicinal and pharmaceutical applications of those preparations.

Earlier we have isolated and characterized a mycelial lectin of the xylotrophic basidiomycete *Grifola frondosa*, strain 0917 [10], that is a hydrophilic dimeric glycoprotein with a molecular mass of about 68 kDa and the protein: glycan ratio of 3:1. In contrast to the extracellular lectins, *L*1 and *L*2, which we have isolated and purified from the culture liquid of shiitake mushroom [11], the *G. frondosa* lectin does not exhibit a specificity to mono-, di-, and amino saccharides, as well as certain glyco derivatives. The hemagglutination caused by lectin is blocked by polysaccharide D-rhamnan with the linear structure of the repeated component →2)-β-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→. In accordance with the classification proposed in work [12], the affinity to specific carbohydrate sequences allows us to define the *G. frondosa* mycelial agglutinin as endolectin. The above D-rhamnan containing a monosaccharide rhamnose in naturally uncommon D-configuration, is one of two O-specific polysaccharides (O-PS) entering a composition of lipopolysaccharide (LPS, or O antigen) of gram-negative soil bacteria *Azospirillum brasilense* Sp245 [13]. It should be noted that according to the chemical analysis data, two O-PS from *A. brasilense* Sp245 strain do not differ in their structure presenting D-rhamnan homopolymer [13], whereas the immunochemical assay reveals differences in the antigen determinants' structure for the given O-PS [14], being in agreement with their distinct capability of interacting with the mycelial lectin from *G. frondosa* mushroom. All the aforesaid enables one to conclude on a rather precise carbohydrate-recognizing specificity of the fungal lectin. In the course of comparing (that is quite acceptable) the above lectin selectivity with the antibodies' selectivity with respect to the said O-PS from azospirilla, of special interest appears the elucidation of immunochemical peculiarities of the lectin; the aim of the present work is just related to the aforesaid.

2. MATERIALS AND METHODS

2.1. Cultures and proteins

Culture of *Grifola frondosa* (Fr.) S.F. Gray, strain 0917 was obtained previously from the basidiomycete collection of the Komarov Botanical Institute (St. Peterburg, Russia), thanks to Dr. N.V. Psurtseva.

Culture of *Lentinus edodes* (Berk.) Singer [*Lentinula edodes* (Berk.) Pegler], strain F-249 was obtained previously from the collection of higher basidial fungi (Department of Mycology and Algology, Moscow State University).

The lectin of *G. frondosa* preparation was obtained as described in the work [10].

The lectins of *L. edodes* preparations were obtained as described in the work [11].

2.2. Homological (specific) antibodies

For obtaining the specific rabbit polyclonal antibodies to fungal lectins, the rabbits were immunized thrice at two-week intervals by sequential injections of 0.5, 1.0 and 1.5 mg of the lectin into their popliteal lymph nodes. During the first immunization, the lectin was mixed in the ratio 1:1 with a complete Freund adjuvant, subsequent immunizations were performed with an incomplete adjuvant. The blood (50-70 ml) from the marginal ear vein was taken in a week after the last immunization. Fractions of immunoglobulins G (IgG) were obtained from antiserum by means of precipitation with ammonium sulphate [16]. IgG concentrations in solutions were detected spectrophotometrically at λ=280 nm, assuming the optical density (absorption) of IgG solution at the l=1 cm optical pathway and 1 mg/ml protein concentration, to be equal to 1.4 [15].

2.3. Non-homological (unspecific) antibodies

In the work, the commercial preparation of human γ-globulin and antibodies against O antigens of the bacteria *A. brasilense* Sp245, S17 and *Sinorhizobium meliloti* P221, obtained as described in the work [16] were applied. Bacterial strains *A. brasilense* Sp245 and S17 possessed different O-specific antigenic determinants and fall into distinct serological groups [17].

For obtaining the conjugate of fungal lectins, as well as of homological antibodies, with colloidal gold particles (15 nm in diameter), the technique [18] was used. The conjugates were stored at 2-4ºC.

2.4. Immunodot assay

For the immunodot assays of interactions of the lectin - colloidal gold conjugate with specific, non-specific polyclonal rabbit antibodies, with the commercial preparation of human γ-globulin, as well as of the protein A - colloidal gold conjugate with the fragments of antibodies, the nitro-cellulose paper strips («Millipore», USA) were used. 2-μl aliquots of the antibodies solutions from the series of twofold dilutions were spotted on nitrocellulose paper strips, fixed at 50ºC for 10 min. To avoid unspecific sorption of the label on nitrocellulosic sites not engaged with samples, the slightly dried paper strips were immersed into 0.15M phosphate-buffer saline (PBS; pH 7.2) containing 0.02% of Tween-20 and 0.1% of polyethylene glycol (PEG-20000), and incubated in this solution for 30 min at stirring. Then the paper strips were rinsed with PBS for 10 min and placed into the solution of lectin- colloidal gold conjugate (or protein A - colloidal gold conjugate in the case of antibodies fragments) for 10-20 min, where the presence or absence of interaction was observed. The paper strips were then rinsed with PBS and dried with the aid of filter paper.

2.5. Fab and Fc fragments of antibiodies

For obtaining *Fab* and *Fc* fragments of antibodies, the antibodies against lectins and the commercial preparation of human γ-globulin were caused to undergo cleavage according to the technique proposed in [19] and adapted to the actual lab conditions. Papain and cysteine-H (0.5 mg each) were inserted in 1 ml-aliquots of solutions of antibodies and human γ-globulin at 5 mg/ml concentrations in 0.1M phosphate buffer (PB). The mixtures were incubated for 4 h at 37ºC, then frozen for 1 h at -20ºC in order to stop a reaction. Then the dialyzed against 0.01M PB mixture was transferred to a column with ion-exchange carrier DEAE-Toyopearl 650M. By means of gradient elution with 0.01-0.3M PB and UV-detection using an Uvicord SII (LKB, Sweden) detector, two fractions were obtained. The first one was Fab fragments, the second Fc fragments with a probable admixture of unbroken antibodies molecules. Applying the dot-analysis of interaction of the antibodies fragments with protein A - colloidal gold conjugate, Fc-fragments were identified.

2.6. Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) of lectins interaction with antibodies was performed

in 96-well polystyrene plates [20]. Mycelial lectin and haptenic O-PS in the case of *G. frondosa*, and L1, L2 lectins at different steps of purification in the case of *L. edodes*, and complex of these substances at various concentrations served as the antigen samples. Hydrogen peroxide with o-phenylenediamine was used as a substrate reagent. The optical density measurements of the tested samples were carried out at 490 nm using an AIF-Ts-01S immunoenzyme analyzer (ZAO ILIP, St. Petersburg, Russia). The concentrations of G. frondosa 0917 lectin (50 μg/ml) and O-PS (200 μg/ml) preparations were selected in compliance with these substances proportion established earlier in the specific reaction «lectin–haptenic carbohydrate».

2.7. Calculation of parameters of lectin binding with antibodies

Using the formulae for the serial dilution method proposed in [21-23] for establishing the parameters of ligand-receptor interaction, as well as the results of ELISA, the constants K_f of formation of *G. frondosa* 0917 lectin complexes with homological and non-homological antibodies were calculated. The method is based on the use of dilution coordinates, and its important advance compared to earlier developed methods consists in the appeared feasibility to determine the searched interaction parameters even in the case of pre-existing ligand-receptor mixture. The principal idea of the above serial dilution method is the application of mass action law equation for establishing the parameters of ligand-receptor interaction not only in the case of using a constant concentration of one of reactants and gradually increased concentration of the other, - those are central for the theory of the Scatchard plot and Klotz plot methods [24, 25], - but also in the case of simultaneous changes in the both reactants concentrations. Therewith the equilibria proposed could be similarly exploited at the synchronous decrease in reactants' concentrations (the simplest way to achieve that is the mixture dilution), as well as at the concentration's synchronous growth [23].

With allowance for the conditions of applying the calculation relationships derived from the mass action law equation and presented by the author in the aforementioned references [22, 23], we calculated the constants of formation K_f for the point of homological antibodies according to the expression:

$$K_f = 1/l\ (A_0/A_i + \sqrt{A_0^2/A_i^2 - d_i A_0/A_i} - d_i). \quad (1)$$

where l is molar concentration of ligand, A_0 is absorption value taken from ELISA at the initial concentrations of ligand and receptor, A_i is absorption value from ELISA at a dilution by d_i times; A_0 and A_i being proportional to molar concentrations of receptor binding centres at the initial concentration and at a dilution, respectively. In the case of non-homological antibodies, K_f was determined in agreement with the equation proposed by the author for monovalent antibodies:

$$A_0/A_i = d_i + lK_f \quad (2)$$

The change in standard free energy ΔG^0 (kJ/mol at 25ºC) is related to the constant of formation by the formula [26]:

$$\Delta G^0 = -RT \ln K_f = -2{,}303RT \lg K_f = -5{,}708 \lg K_f \quad (3)$$

where R is a gas constant, T is absolute (Kelvin) temperature. The quantities of changes in standard free energy ΔG^0 of the lectin complexes with antibodies were calculated using the average values of logarithms of the formation constants obtained.

3. RESULTS

Carbohydrate-binding capabilities of the lectins was tested by the method of hemagglutination inhibition. The possibility of using erythrocytes of various types in the hemagglutination reaction was analyzed during selection of optimal conditions. The trypsinized rabbit erythrocytes were chosen as the most sensitive test object for the *L. edodes* lectins.

The rabbit polyclonal antibodies against the homogeneous preparations of the mushrooms *G. frondosa* (Fr.) S.F. Gray mycelial lectin and *L. edodes* extracellular lectin *L*1 were obtained, and the immunochemical specificity of their interaction with the antigenic preparation demonstrated. That antibodies - antigen (lectin) interaction was confirmed by means of immunodot, ELISA

and immunodiffusion methods. Surprisingly, the immunodiffusion assay in the case of *G. frondosa* showed a lack of specific interaction. Antibodies against O antigens of the bacteria *A. brasilense* Sp245 and S17, as well as a commercial preparation of human γ-globulin, all used in dot-analysis as negative references, revealed a cross-reaction toward this lectin conjugated with colloidal gold particles (Figs. 1, 2).

Applying the treatment by proteolytic enzyme papain and ion-exchange chromatography, the antigen-binding fragments (*Fab* fragments) of antibodies against the mycelial lectin and human γ-globulins used as references were obtained. *Fab* fragments of antibodies were examined in respect to their interaction with antigen under study, and a positive response was revealed solely for *Fab* fragments of antibodies obtained against the mycelial lectin (Fig. 3).

The ELISA results have demonstrated the presence of common antigenic determinants in the composition of two *L. edodes* extracellular lectins (Fig. 4). At the first step of purification [11] the lectin 2 occurs in supernatant over the solid phase (just crude lectin 1) precipitated using acetone from the culture liquid filtrate. This crude *L*2 capability of interacting with the anti-*L*1 antibodies appears to be about one order of magnitude greater (judging from the A_{490} values, Fig. 4) than the corresponding quantity for crude *L*1. Only in the process of further purification, the lectin 1 becomes more superior as compared to lectin 2 in this context (see 1.0 and 2.0 notations, respectively, in Fig. 4).

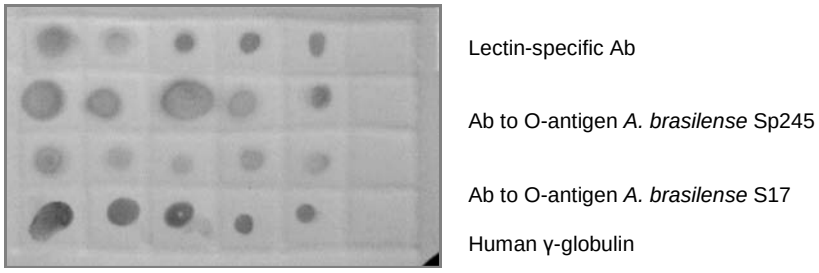


Fig. 1. Interactions between *G. frondosa* 0917 lectin labeled with colloidal gold and various antibodies (Ab).

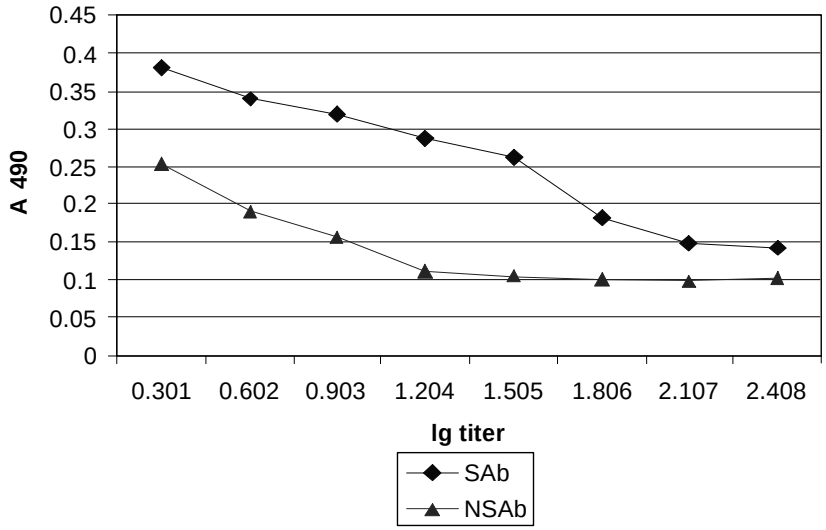


Fig. 2. Interactions between G. frondosa 0917 lectin and specific (SAb) and non-specific (NSAb; against O antigen *Sinorhizobium meliloti* P221) antibodies detected by ELISA.

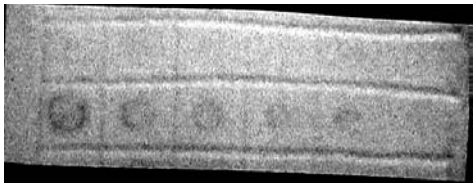


Fig. 3. Interactions between colloidal gold-labeled fungal lectin and antigen-binding domains of the antibodies (*Fab*-fragments).

Table 1. The K_f -parameters of *G. frondosa* lectin binding with specific (SAb) and non-specific (NSAb) antibodies calculated on basis of ELISA data.

Lectin M_r = 67 kDa [10]		Absorption in ELISA[a]		$K_f \times 10^8$ m^{-1}	
Absolute concentration, μg/ml	Molar concentration, $\times 10^{-9}$ m	SAb	NSAb	SAb	NSAb
200	2.941	0.381	0.253	4.97	2.83
100	1.471	0.339	0.190	4.54	2.41
50	0.735	0.318	0.157		
25	0.368	0.276	0.110	5.16	3.15

[a] Data averaged over not less than 15 repeats.

Constants K_f of complex formation of the mycelial lectin with specific and non-specific (to O antigen of bacterium *Sinorhizobium meliloti* P221; randomized choice) antibodies, calculated by the ELISA data are shown in Table 1.

The average value of change in standard free energy ΔG^0 on the binding formation «*G. frondosa* 0917 lectin - homological antibodies» calculated by the equation (3) was equal to -49.597 kJ/mol at 25ºC; ΔG^0 of the complex «*G. frondosa* 0917 lectin - non-homological antibodies» was about -48.216 kJ/mol at 25ºC.

4. DISCUSSION

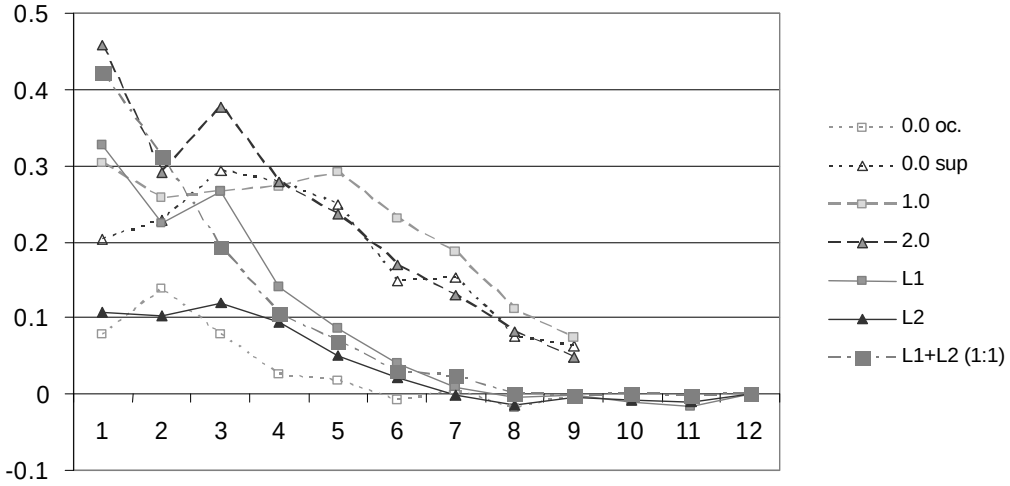
Commonly recognized high specificity of "antigen-antibody" recognition is a kind of biological postulate. However, various lectins are noted to be capable of bonding also non-specific immunoglobulins [27] *via* oligosaccharides incorporated in the latter; with the allowance for the fact just mentioned, a term of antigenicity should be regarded as unclear. In spite of that, the lectin's ability for non-specific binding with antibodies is not a paradox due to the uniform principle of biomolecular surfaces complementarity central for all the aforesaid intermolecular contacts. Since those substances are involved in biosystems of actual interest, in our opinion, establishing the parameters of their interaction seems to be important and aimed at deeper insight into the phenomenon of bilateral biospecificity of the lectin.

4.1. *Lentinus edodes* extracellular lectins

The experiments conducted to see whether antibodies raised against *L*1 protein from *L. edodes* F-249 would react with *L*2 used as purified preparation, in crude culture liquid and in artificial mixture *L*1+*L*2, have led us to the following indications. There are few antigenic determinants in common between the two

extracellular lectins of shiitake, therewith crude *L*2 is much more effective in binding the anti-*L*1 antibodies compared with not only purified *L*2, but also the crude *L*1 as itself (see Fig. 4). It seems reasonable to suppose that crude *L*2 being admixed with *L*1 *in vivo*, i.e. in culture liquid, presumably displays the more profound reaction with antibodies raised against one of them, *L*1. The more so that our earlier observations [29] revealed the considerable mutual influence of *L*1 and *L*2 with respect to the change in their lectin activity: hemagglutination titer increased from 16 (for either of two proteins in diluted buffer solutions) up to 1024 (i.e. by 128 times) when the lectins' solutions mixing (1:1, v/v) and incubating at 26°C for 30 min. Obviously, the spatial

a)



b)

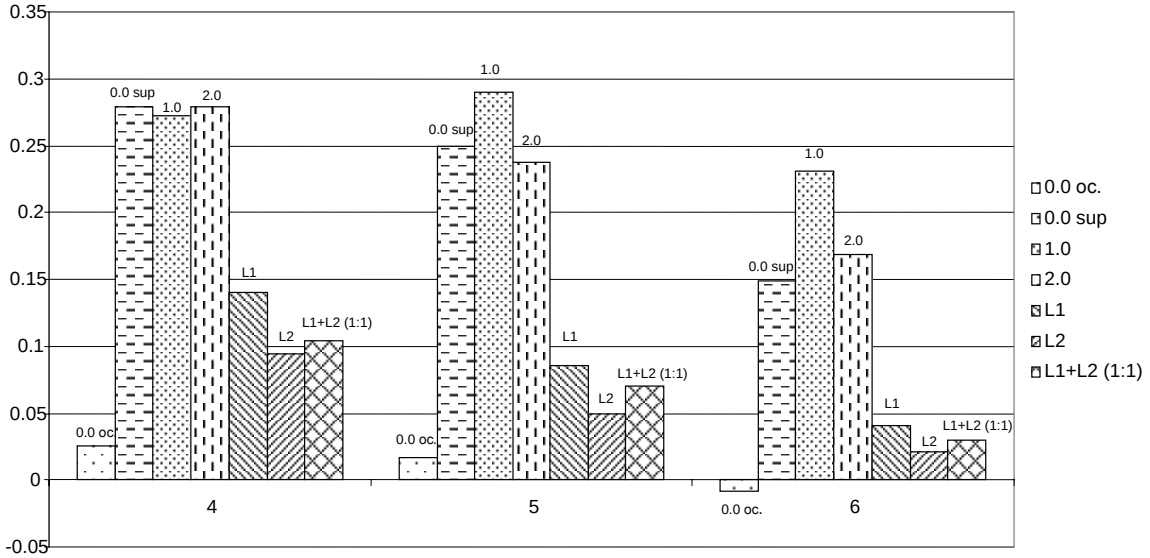


Fig. 4. ELISA-detected interactions between *L. edodes* extracellular lectins and antibodies raised against *L*1: a) as introduced into microtiter wells in a series of double dilutions (abscissa); b) as a diagram constructed for linear parts of (a) curves, where 0.0 oc, 1.0 and *L*1 are the successive steps of lectin 1 purification; 0.0 sup, 2.0 and *L*2 are the successive steps of lectin 2 purification; Oy axis is for A_{490} values.

arrangement of *L*1 + *L*2 complex assists greater accessibility of carbohydrate-binding sites, in the given case of the glycoconjugates binding on the test erythrocytes surface. Nevertheless, the Figure 4 shows that mixing the lectins preparations to form *L*1+*L*2 could not result in the high-selectivity (crude *L*2 toward non-specific antibodies) phenomenon mentioned just above.

And what occurs with the shiitake lectins' selectivity in respect to carbohydrate structure binding during the process of proteins purification? The reaction of hemagglutination is based on specific binding of lectins with carbohydrates present on the surface of erythrocytes; therefore, it is possible to judge their carbohydrate-binding properties by the character of agglutination of erythrocytes by lectins. Erythrocytes from various organisms differ from each other in carbohydrate dominants. We tested the agglutinating properties of *L. edodes* extracellular lectins of different purification rate using native and trypsin-treated erythrocytes of rabbit, cow, sheep, horse, and humans (of the four blood groups). Both lectins gradually become sensitive not at all to the human erythrocytes of four blood groups, in spite of initially high hemagglutination titers in the system "lectin 2+native I (O) blood group erythrocytes" and "lectin 2+trypsinized IV (AB) blood group erythrocytes" (1024 and 512, respectively). Apparently, the loosely-bound low-molecular admixtures (released *via* the Sephadex G-25 treatment [11]) facilitated the crude lectins interaction with human erythrocytes. Purified preparations did not cause the agglutination of human erythrocytes. The same is for the trypsin-treated rabbit erythrocytes, the extent of complementarity to which increased by 64 and 2 times in the course of purification of *L*1 and *L*2, respectively.

Thus, the purified extracellular lectins of *L. edodes* displayed high selectivity on recognition of definite structures on the surface of trypsinized rabbit erythrocytes. The absence of agglutination reaction when using the erythrocytes other than rabbit and human, was shown. Hemagglutination titer 4 with sheep blood cells seems to be neglected compared with the values of 256 to 32800 with rabbit erythrocytes. The treatment of erythrocytes with trypsin significantly increased the sensitivity of the reaction (by 8 times for both purified lectins), that is commonly explained by the higher accessibility of lectin receptors on the trypsinized erythrocytes' surface compared to native cells. One can suggest therefore that the binding properties of lectins *in vivo* complicated by appropriate admixtures exert the system “lectin- non-specific antibody” characteristics to be opposite to those with isolated and purified lectin preparations. That is obviously due to the common hapten, carbohydrate specific to the given lectin, is a component of fungal culture medium and of antibodies raised against the lectin.

4.2. *Grifola frondosa* mycelial lectin

Comparative description of the ELISA-detected lectin's interaction with (i) specific and (ii) non-specific antibodies have shown that in the case (i), the decrease in lectin concentration leads to occurrence of steadily weakening interaction with antibodies, the 32-times dilution of the protein being characterized by the absorption range as low as from 0.4 down to 0.25 for *G. frondosa* lectin. In the case (ii) of non-specific antibodies, a decrease in mycelial lectin concentration by 8 times results in sharply weakened interaction altering from maximal to minimal level. For the purposes of confirming the immunochemical specificity of the lectin interaction with homological antibodies, protolytic cleavage of the latter along with human γ-globulins as a kind of negative reference was carried out. Taking into account the *Fab* fragments of human γ-globulins bounding to the lectin, one could accept as a proved fact that antigen-binding centre of γ-globulin molecules does not involve in their interaction with the lectin. Namely the lectin-carbohydrate recognition, when the selected oligosaccharides involved in the antibodies’ carbohydrate moiety interact with the competent pool of fungal lectin, appears to be basic for interaction between the *Fc* fragments of non-specific immunoglobulins and the fungal lectin under study.

Quantitative characteristics of intermolecular interactions including those between carbohydrate and protein are known to be an equilibration constant K_f and a change in standard free energy

ΔG^0, therewith the more negative is the ΔG^0 value, the stronger is the bond. The K_f quantity varies with the extent of the interacting molecules mutual affinity. It is clear that the term “affinity” adopts distinct meanings in the differing cases of the lectin interaction with specific antibodies («antigen-antibody») and with non-specific antibodies («lectin-carbohydrate»). In the former case of specific antibodies, the parameters were calculated by means of equation (1) starting from the conclusions made by the author of the method [24]. This equation proposed for bivalent antibodies is valid for the antibodies homological то the lectin, those being bivalent. The evidences are following: the existence of two centers for specific antigen binding in accordance with the antibodies’ molecular structure theory; the experimentally confirmed occurrence of specific binding of the lectin under question with homological antibodies. In the case of non-specific antibodies, the parameters were calculated by means of equation (2) starting from the unlikely feasible involvement of more than one “site” in the lectin-antibody interaction based on «lectin-carbohydrate» bonding. For the same reason, molar concentration of the lectin (ligand) *complex* with non-homological antibodies (receptor) could be assumed very likely to be incomparably lower in respect to the *purified lectin* molar concentration, thus the condition of equation (2) applicability becomes fit. The constants of binding (formation) for the interacting substances in dynamical equilibrated system obtained in compliance with the equations (1) and (2), taking into consideration the twofold sample titration used in ELISA, are quantitative characteristics of the lectin as antigen. The data presented in Table 1 show that the values of constants of the lectin binding (at equal molar concentrations) with homological antibodies are about 2 times higher compared with non-homological antibodies. In spite of that, the values of changes in standard free energy ΔG^0 are essentially the same, testifying to near-equal strength of the complexes thus formed, even though the molecules affinity differs qualitatively. Consequently, the sites of lectin binding to homological and non-homological antibodies are non-coincident. With allowance for the value of approximately 5 kJ assigned to the formation of one hydrogen bond [26], the contact of lectin molecules with antibodies could be assumed to be provided by about 10 bounding OH-groups.

The ligand-receptor association controlled by a large number of factors undergoes the effects related to cellular responses for changes in any external factor. According to the work [2], a principal contribution to Gibbs free energy on «lectin-carbohydrate receptor» binding is made due to the hydrogen bonds formation, where both donors and acceptors of electrons are hydroxyl groups from a carbohydrate-binding pocket of the lectin and haptenic polysaccharide. Obviously, a high specificity of such associations as «lectin - receptor carbohydrate» and «antigen - antibody» is provided by the most perfect complementarity in respect to the spatial topology of bound groups, thus the structural peculiarities of contacting biomolecules are of ultimate importance. Antibodies secreted by B-lymphocytes vary severely by the carbohydrate moiety composition, depending upon the assigned class of immunoglobulins, as well as upon the given B-lymphocytes producer - microorganism at all [28]. The above mentioned carbohydrate specificity of the fungal lectin have brought us to searching for information on rhamnan chains incorporated in oligosaccharides of rabbit immunoglobulins, yet being unsuccessful.

We consider the following most reasonable explanation of the data obtained. As an endolectin, the *G. frondosa* 0917 lectin is sensible to polysaccharide conformational modifications caused primarily by glycosidic bonds and monosaccharides configuration. The extremely sensitive binding is characterized by numerous bonds, mainly hydrogen ones, and depends severely on the spatial availability of bounded groups in the target carbohydrate. With the other conditions being equal (generally, the quantity of useful bonds of the lectin “pocket” with sugar OH-groups, the latter’s spatial accessibility dependent on the polysaccharide’s glycosidic bond), the variations in one or two OH-groups and protons at the asymmetrical carbon atoms, inherent in the majority of glycopyranoses properties, could be apparently treated as playing non-decisive role in lectin recognition. Therefore,

limiting factors for endolectins recognition should be the definite glycosidic bonds of a target carbohydrate and the conformational accessibility provided by them for the *specific moiety* of reactive OH-groups resulting in the *specific moiety* of bonds. We assume that if the aforesaid moiety of newly formed bonds remains the same on the lectin interaction with different macro-receptors, the highly specific recognition occurs. *Specific moiety* of the receptor polysaccharide plays a decisive role here in compliance to the explicit interaction of the lectins (under our study and some others) with so distinct macroreceptors naturally comprising the distinct oligosaccharide compositions, as human and rabbit immuno-globulins against the absolutely different antigens. Possibly, our experimental results under discussion display essentially the same values of energy of the lectin-antibody interactions (strength of complexes), in spite of distinguished affinity of the lectin toward antibodies originated from variable sources. Nevertheless, the homological antibodies characterized by the greater binding constants values are more successful in competitive reactions to form bonds with the lectin, as compared with the non-homological antibodies.

The availability of antibodies to the lectin *L*1 of *L. edodes* F-249 made it possible to test the degree of antigenic similarity among the lectins *in vivo* and *in vitro*. The results presented here indicate that while antibodies raised against *L*1 lectin will strongly react with culture liquid (first of all the *L*2 lectin component of that), it will react only slightly with the *L*2 preparations purified from culture liquid. The non-specificity of antibodies against the fungal lectin appears to be manifested *in vitro* only.

The discussion presented is concerned with the isolated substance-level assays. Probably we couldn't transform exactly the results obtained at a molecular level toward the knowledge on biological specificity of macromolecules at the level of living tissue. The lectins are potentially capable of revealing the unambiguous carbohydrate specificity, therefore the results of *in vitro* assays using lectins should be interpreted carefully when concluding on their behaviour in living systems.

ACKNOWLEDGMENTS

We thank our colleagues from the Institute of Biochemistry and Physiology of Plants and Microorganisms of the RAS: Prof. V. A. Bogatyrev for help in marking the lectin with colloidal gold; Prof. S. Yu. Shchegolev and Dr. E. E. Fedorov for valuable discussion.

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