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EXPERIMENTAL ARTICLES

Relationship between the Molecular Structure of the Nitrogen Source and the Activity of the Extracellular Lectins of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] upon Submerged Cultivation

O. M. Tsivileva*, A. N. Pankratov, V. E. Nikitina*,[1], and L. V. Garibova*****

**Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, pr. Entuziastov 13, Saratov, 410015 Russia*
***Department of Analytical Chemistry and Chemical Ecology, Faculty of Chemistry, Saratov State University, ul. Astrakhanskaya 83, Saratov, 410026 Russia*
****Department of Mycology and Algology, Faculty of Biology, Moscow State University, Vorob'evy gory, Moscow, 119992 Russia*

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Abstract—The activity of the extracellular lectins of *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler] and the formation of a pigmented mycelial film by this fungus upon submerged cultivation in a synthetic medium were found to depend on the presence of some amino acids (particularly, asparagine) and Ca^{2+} and Mn^{2+} ions in the medium. Quantum-chemical calculations suggest that the different character of the interaction of amino acids with the aforementioned ions is due to differences in the hydrophobicity of the amino acids rather than to differences in the electron structure of the amino acid zwitterions.

Key words: lectins of higher fungi, submerged cultivation, *Lentinus edodes*, amino acids, molecular structure, quantum chemistry.

There is little information in the literature on the lectins of the shiitake mushroom, *Lentinus edodes* (Berk.) Sing [*Lentinula edodes* (Berk.) Pegler]. In particular, Jeune *et al.* described a mitogenic lectin isolated from the fruiting bodies of this mushroom [1]. Earlier, we investigated the effect of some factors (the composition of the nutrient medium and the morphogenetic stage of the mushroom) on the lectin activity of *L. edodes* [2, 3]. Of particular interest are experimental data on lectin activity at the stage of formation of various vegetative structures, such as the brown mycelial film produced by the shiitake mushroom [4].

This work was undertaken to study the relationship between the activity of the extracellular lectins of *L. edodes*, the formation of a mycelial film in the submerged culture of the mushroom, and the type of nitrogen source in a synthetic cultivation medium.

MATERIALS AND METHODS

The strain *Lentinus edodes* F-249 used in this study was obtained from the collection of higher basidial fungi of the Department of Mycology and Algology, Faculty of Biology, Moscow State University. The strain was maintained on malt extract agar at 4°C. Upon submerged cultivation, the strain was grown in a synthetic medium containing 50 mM glucose as the carbon source, ammonium chloride or sodium nitrate as the nitrogen source (the carbon-to-nitrogen ratio in the medium varied from 7.5 : 1 to 150 : 1), and some essential amino acids (20 mM with respect to the nitrogen concentration). The medium was also supplemented with Ca^{2+} or Mn^{2+} ions in the form of chlorides. The cultivation temperature was 26°C, which is optimal for *L. edodes* [5].

Liquid nutrient media were inoculated with brewer's agar blocks (3 blocks 5-mm per 50 ml of the medium) cut from the zone of shiitake growth [6].

The hemagglutinating activity of the culture liquid was determined by using a 2% suspension of trypsinized rabbit erythrocytes [7]. The hemagglutination titer (HAT) was defined as the highest dilution of the culture liquid causing detectable hemagglutination.

Ab initio calculations by the Hartree–Fock method (basis 6-31G*) [8] were performed with the aid of the HyperChem software package (Hypercube, Inc., Gainesville, Florida, USA) in terms of the Polack–Rieber algorithm [9]. The gradient norm during quantum-chemical calculations did not exceed 0.02 kcal/(mol Å).

The data obtained were statistically processed by the standard methods.

[1] Corresponding author.

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RESULTS AND DISCUSSION

The involvement of lectins in the formation of a mycelial film by *L. edodes* F-249 was revealed in our previous study [3], which showed that the higher the lectin activity of the *L. edodes* F-249 mycelium, the higher the formation rate of the mycelial film. When *L. edodes* F-249 was grown on brewer's wort agar or an oak sawdust–grain substrate, lectin activity increased at the stage of mycelial film formation and decreased at the stage of formation of primordia and fruiting bodies. The formation of the mycelial film was accompanied by a drastic rise in the hemagglutination titer of extracts from primordia and fruiting bodies [3].

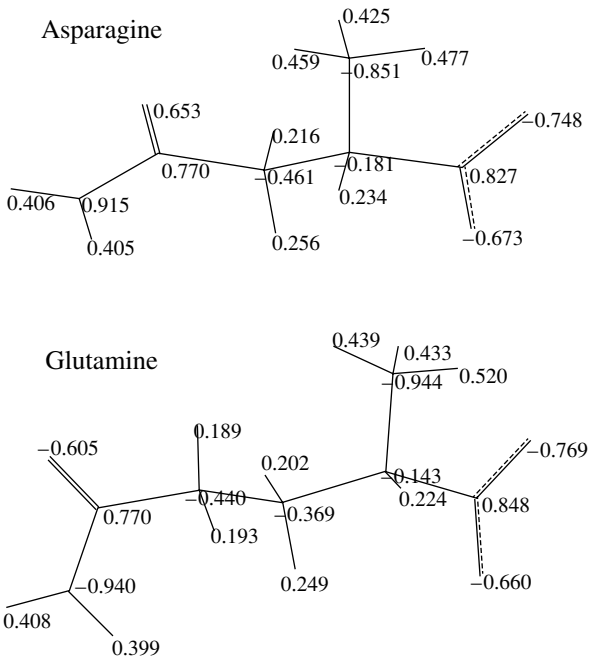
In this study, we attempted to initiate the formation of a mycelial film by *L. edodes* F-249 grown in liquid synthetic media. For this purpose, we optimized the nutrient medium of the fungus in order to enhance its lectin activity. Tables 1 and 2 show how different molar proportions of carbon and inorganic nitrogen sources in the medium influence the hemagglutination titer of the culture liquid. As can be seen from these tables, the lectin activity of the culture liquid of *L. edodes* F-249 was maximum when the carbon-to-ammonium molar ratio in the nutrient medium was 150 : 1.

Similar experiments with amino acids as nitrogen sources (Table 3) showed that the activity of the extracellular lectins of *L. edodes* F-249 was at a maximum (the HAT of the culture liquid was 4096) when the C : N ratio in the synthetic nutrient medium was 17 : 1. In this case, however, the mycelial film was formed only after 55–60 days of cultivation.

We attempted to accelerate the formation of the mycelial film in the synthetic medium and found that this formation could be accelerated by the addition of Ca^{2+} ions at concentrations of 2–10 mM or Mn^{2+} ions at concentrations of 0.5–2 mM to the amino acid–containing synthetic medium. The enhancing effect of these ions was absent if the medium did not contain amino acids.

Among the different amino acids studied, asparagine (in the presence of either Ca^{2+} or Mn^{2+} ions) was found to exert the most profound effect on the mycelial film formation in submerged *L. edodes* F-249 cultures. This suggests that asparagine may be involved in some calcium(II)– or manganese(II)–mediated biochemical processes. If so, asparagine must differ from other amino acids (even those that are structurally close) in electronic structure. On the other hand, it is known that lectins interact with carbohydrates through the formation of numerous hydrogen bonds and that the hydrogen atom of the amide group and the oxygen atom of the carboxyl group of asparagine are involved in the lectin–carbohydrate interactions [10].

The suggestion that the asparagine molecule reversibly interacts with bivalent cations via the primary amide group rather than chelates such cations with the involvement of the amino and carboxyl groups is confirmed indirectly by the fact that the formation of chelate complexes is more known for copper(II) than for calcium(II) and manganese(II) [11]. Due to conjugation, the nucleophilic properties of nitrogen atoms in amide groups are diminished, and the most likely candidate for the binding of metal cations is the oxygen atom. This follows from the idea of hard and soft Lewis acids and bases [12], according to which calcium(II) and manganese(II) cations (hard Lewis acids) interact preferentially with a hard base—the oxygen atom. In the case of a reactive COOH group, the binding of metal cations to the oxygen atom has no alternative. The interaction of hard acids and bases is controlled by charges [12].



Scheme showing the distribution of charges over the atoms of the asparagine and glutamine zwitterions calculated by the RHF/6-31G* method in terms of Mulliken's orbital population density [8].

Glutamine is the closest structural analog of asparagine with the $CONH_2$ group in the molecule. In spite of the fact that the glutamine and asparagine molecules differ only in one methylene group, the presence of glutamine in the cultivation medium of *L. edodes* F-249 little influenced the formation of the mycelial film of the shiitake mushroom. Taking into account the foregoing, the difference in the action of these amino acids can likely be explained by different charges on their probable reaction centers.

In aqueous solutions, amino acids occur as zwitterions. Bearing this in mind, we computed the electronic structure of the asparagine and glutamine zwitterions by the restricted Hartree–Fock method (basis 6-31G*) [8]. The results are presented in the figure as charges on the atoms constituting the asparagine and glutamine molecules. These charges determine the reactivity of substances in the reactions governed by electrostatic

Table 1. The effect of the carbon-to-nitrogen ratio in the synthetic nutrient medium on the hemagglutination titer of the culture liquid of *L. edodes* F-249 grown in a submerged mode with NH_4Cl as the nitrogen source

C : N				
150 : 1	75 : 1	30 : 1	12.5 : 1	7.5 : 1
1024 (13)	64 (1, 9–27)	64 (1, 13–27)	64 (13–27)	64 (13–27)

Note: Parenthesized is the age (days) of the *L. edodes* F-249 culture.

Table 2. The effect of the carbon-to-nitrogen ratio in the synthetic nutrient medium on the hemagglutination titer of the culture liquid of *L. edodes* F-249 grown in a submerged mode with $NaNO_3$ as the nitrogen source

C : N					
150 : 1	75 : 1	30 : 1	15 : 1	10 : 1	7.5 : 1
512 (1–3, 7, 11–14)	512 (7, 11)	128 (1–3, 7–17)	128 (1–3, 7, 11–17)	512 (3)	256 (3)

Note: Parenthesized is the age days of the *L. edodes* F-249 culture.

Table 3. The effect of the carbon-to-nitrogen ratio in the synthetic nutrient medium on the hemagglutination titer of the culture liquid of *L. edodes* F-249 grown in a submerged mode with L-asparagine as the nitrogen source

C : N					
152 : 1	77 : 1	32 : 1	17 : 1	12 : 1	9.5 : 1
128 (1–9)	1024 (3–7)	1024 (1–7)	4096 (3–7)	1024 (3–7)	1024 (1–7)

Note: Parenthesized is the age days of the *L. edodes* F-249 culture.

forces, to which the hard–hard interactions of asparagine and glutamine with Ca^{2+} and Mn^{2+} cations belong.

As can be seen from the figure, the charge characteristics of the plausible reaction centers of the asparagine and glutamine zwitterions are very similar and cannot explain the difference in the interaction of these amino acids with the metal cations.

To reveal other factors that may determine the chemical behavior of the two amino acids in their reactions with the metal cations, we calculated the quantitative structure–activity relationships (QSARs) for the asparagine and glutamine zwitterions with the aid of the HyperChem software package (Table 4).

The results presented in this table show that the glutamine molecule is characterized by higher values of the van der Waals surface area [13–15] and volume [15], refractivity [16, 17], polarizability [18], and $\log P$ (P is the distribution index in the 1-octanol–water system, which is a measure of hydrophobicity [16, 17, 19, 20]) than the asparagine molecule. It should be noted that the hydrophobicity of asparagine and glutamine molecules is moderate, as is evident from a comparison of their $\log P$ values (1.76 and 2.01, respectively) with those of highly hydrophobic hexane and 1-octanol (2.88 and 2.53, respectively) and of highly hydrophilic methanol and water (–0.27 and –0.51, respectively).

The difference in the reactivity of the asparagine and glutamine zwitterions could be related to their different hydrophobicities. Indeed, asparagine is less hydrophobic than glutamine. Consequently, the asparagine molecule must be more hydrated and, hence, must bind metal cations less tightly than does the glutamine molecule. It should be noted, however, that the calculated hydrophobicities of asparagine and glutamine differ insignificantly (Table 4). Moreover, the atomic additive scheme of the $\log P$ calculation does not take into account the high degree of charge separation in molecular systems and, hence, underestimates their hydrophilicity and the tendency (due to the similarity of their electron density distributions) toward leveling off of the hydrophilicity (figure). The differences in the polarizability and refractivity of the asparagine and glutamine molecules are not of crucial importance since hard–hard interactions are mainly electrostatic [12].

The higher reactivity of asparagine as compared with that of glutamine may also be related to a spatial factor. If so, the topologically appropriate fragments of protein molecules can be found based on the calculated values of their van der Waals surface area and volume, as well as on the data of x-ray analysis.

Thus, the lectin activity of *L. edodes* F-249 grown in synthetic nutrient medium depends on the simultaneous

Table 4. Some calculated QSAR parameters for the asparagine and glutamine zwitterions

Amino acid	van der Waals surface area according to ([13, 14]), Å^2	van der Waals surface area according to ([15]), Å^2	van der Waals volume, Å^3	$\log P$	Refraction, Å^3	Polarizability, Å^3
Asparagine	245.78	283.20	407.54	1.76	23.20	10.59*
Glutamine	286.46	314.73	464.69	2.01	27.96	12.43*

* The atomic additive scheme of calculations included no parameter for the ammonium nitrogen atom.

presence of asparagine and Ca^{2+} and Mn^{2+} ions in the medium. The mycelial film of the shiitake mushroom grown in a submerged culture is formed in a few days. The ab initio quantum-chemical computations by the RHF/6-31G* method showed that the different character of the interaction of asparagine and glutamine with metal cations cannot be related to differences in the electronic structure of the amino acid zwitterions. At the same time, the calculated QSAR parameters of the zwitterions suggest that the observed difference in the reactivity of asparagine and glutamine may be related to their different hydrophobicities. The data obtained allow the suggestion to be made that asparagine influences the formation of the mycelial film of *L. edodes* F-249 cultivated in synthetic medium due to its involvement in biosynthetic processes rather than due to the chemical binding of Ca^{2+} and Mn^{2+} ions.

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