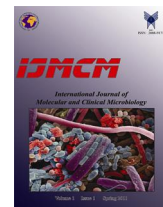


# International Journal of Molecular and Clinical Microbiology



## Review

## Lectin and enzyme relationships in microbiology

Mikhail Lakhtin<sup>a,\*</sup>, Vladimir Lakhtin<sup>a</sup> and Vladimir Alyoshkin<sup>a</sup>

<sup>a</sup> Institute: G.N. Gabrichevsky Research Institute for Epidemiology and Microbiology, Russia

### ARTICLE INFO

#### Article history:

Received 22 November 2010

Accepted 7 February 2011

Available online 11 April 2011

#### Keywords:

Lectin, enzyme, co-functioning

### ABSTRACT

Examples of inter- and intramolecular direct co-functioning of lectins and enzymes are summarized. The data indicate importance of lectin-enzyme relationships in molecular and clinical microbiology and medical biotechnology. Lectin-enzyme relationships have extended prospects in medicine and biotechnology.

## 1. Introduction

Lectins (carbohydrate recognizing proteins) are widely distributed in nature (Lakhtin, 1987; Sharon and Lis, 2003; Lakhtin et al., 2010a). They are distributed as co-functioning systems, and represent universal objective for molecular microecology (Lakhtin et al., 2010b; Lakhtin et al., 2010c). Studies in the field of lectins and enzymes are quickly developed and converged. The aim of this review is to give briefly current views on relationships between enzymes and lectins, and their prospects in future.

### 1.1 Lectin Terms

There are a number of important key terms concerning lectin origin, determination, specificity, organization and functioning, which may be taken into consideration when modern current knowledge on lectins is analyzed (Lakhtin et al., 2010d).

Briefly, term “lectin” means:

I) the presence of carbohydrate/glycoconjugate-binding site (CBS) or configuration of non-catalytic nature:

II) Interaction without altering recognizable carbohydrate;

III) Reversible carbohydrate recognition;

IV) inhibition by complementary carbohydrate or glycoconjugate;

I) non-immunoglobulin nature;

II) Ig-like domain/motif can be presented, and may be important in pair together with CBS.

I) non-toxic nature of CBS:

II) as opposite term to toxic moiety of molecule like in case of counter pair: enzyme and lectin activities in A (toxic enzyme) - or B (non-toxic lectin)- subunit of A-B-type toxins, respectively.

The term “enzyme as true lectin” means the presence of enzyme and lectin activities in different separate positions in the same molecule (different subunits, epitopes, domains, etc)

\*Corresponding author: Vladimir Lakhtin  
Tel: 007 495 452 1816; Fax: 007 495 452 1830  
E-mail address: lakhtinv@yandex.ru

(see Table 2). Historically, examples of lectin-enzyme relationships were described and accumulated preferentially as intermolecular followed by intramolecular type.

### 1.2. Intermolecular Lectin-Enzyme Relationships

In the Table 1 the data on successful testing glycoprotein origin of about 500 enzymes (and their protein modulators) from all classes are presented. As a result of lectin-enzyme interaction, modulation, stabilization, and space orientation of enzymes are reached (Lakhtin et al., 2010c).

These data demonstrate high potential for intermolecular enzyme – lectin relationships (lectin-directed enzyme stereo fixation, stabilization, assembling, modulation, etc.).

### 1.3. Intramolecular Lectin-Enzyme Relationships

Molecular organization of lectins often includes receptor part/fragment/domain/motif of molecule (glycoconjugate binding) in combination with enzyme moiety of the same molecule (Lakhtin, 1994). Up today, the progress in the field of intramolecular lectin-enzyme natural relationships is impressive. Some examples are presented in Table 2.

At present it is clear that all main classified types of enzymes can be represented by true lectins.

It should be noted that the same carbohydrate recognition sites/motifs are often found in proteins including different classified enzymes. For example, Gal-binding domain-like is revealed not only in galactose oxidase but also in tyrosine protein kinase, *Lactococcus* X-Pro dipeptidyl-peptidase, lysyl endopeptidase, and nicotine adenine dinucleotide glycohydrolase (EBI 2010 > Databases > InterPro.IPR008979). Besides, the same CBM type can be found among archaea, eubacteria and fungi as well (Abbott et al., 2008).

Tables 1 and 2 show that lectin – enzyme relationships are universal ones in any organism. They are involved in any host-bacteria symbiotic or non-symbiotic processes, receptor recognition, cytoplasm-organelle metabolism, etc. For instance, lectins and enzymes are active co-partners in eukaryotic intracellular glycoprotein modification and degradation systems (Yoshida, 2007). Their

co-functioning is of importance for any biological process in vitro and in vivo as well as for industrial biotechnology (Lakhtin et al., 2010c).

Aforementioned above data indicate importance of lectins and their intra- and interrelationships with enzymes in regulating metabolic nets in vivo and in vitro.

### 1.4. Probiotic Microbial Lectins

Among lectin sources, probiotic microbes are of increased interest. Probiotic microbial strains of human origin are important perspective sources of bioactives and useful metabolites for drug forms (Aleshkin et al., 2008; Lakhtin et al., 2008). Among such metabolites, there is a variety of lectin – enzyme potentially co-functioning systems involving in recognition type interactions between probiotic microbes and the host (Lakhtin et al., 2006b; Lakhtin et al., 2010c) (Table 2). The researchers are investigating lectins of probiotic bacteria species and strains (*Bifidobacterium adolescentis* MC-42, *B. bifidum* 1, *Lactobacillus acidophilus* NK1, K3III24, 100ash, and *L. plantarum* 8RA3) – key ingredients of probiotics and synbiotics in Russia (Shenderov, 2008). Isolated and standardized on their properties lectins of probiotic lactobacilli and bifidobacteria have revealed different useful synergistic properties (anti-pathogen, etc.) (Lakhtin et al., 2006a, Lakhtin et al., 2010a, Lakhtin et al., 2010b). It is clear now that probiotic multistrain lactobacillus consortium Acilact possesses three different lectin systems involving in different protection reactions by different mechanisms (Lakhtin et al., 2011).

It is waiting for extended panel of probiotic microorganism glycoconjugate-recognizing activities at the level of:

- truncated lectin molecules (in the presence of hydrolases of surrounding media when lectin activity is become unmasked, increased, or changed);

- lectin complexes and multimere assemblies when expression of new type of glycoconjugate recognition is possible (which is absent at the level of monomer ingredients);

- lectin-like molecular pool (bacteriocin-like substances which can be selectively and reversibly associated with glycoconjugates; etc).

**Table 1**

Typical selected examples on interactions between classified enzymes (also their protein modulators) from about 450 taxonomic sources and extended panel of carbohydrate sensitive agents (mainly commercial lectins) (Lakhtin M et al., 2010c).

EC*	Enzymes	Interaction with carbohydrate sensitive agents
1.	Oxidoreductases	57
2.	Transferases	78
3.	Hydrolases	330
3.1.	Esterases	75
3.2.	Glycosyl hydrolases	68
3.4.	Peptidyl hydrolases	148
3.5. - 3.10.	Other hydrolases	39
4.	Lyases	14
5.	Isomerases	6
6.	Ligases	5

\*EC according to International Enzyme Classification (Enzyme Nomenclature Recommendations 1992. Orlando: Acad. Press. 1992).

**Table 2**

Examples of bifunctional enzymes with intrinsic non-catalytic lectin properties

EC, Enzymes (sources)	Carbohydrate-binding* domains, motifs, etc.	References
1.1.3.9. Galactose oxidase (Fungi)	Galactose-binding motif	Ito et al., 1991
2.4.1.-. UDP-GalNAc:Polypeptide $\alpha$ -N-Acetyl-GalNAc-transferase-2 (human)	lectin domain: 3 sites	Fritz et al., 2006
2.4.2.30. ADP-ribosyltransferases ( <i>Bacillus</i> , <i>Clostridium</i> )	Domains in B-subunit of A-B toxins	Barth et al., 2004
2.7.-.-. Protein kinases ( <i>M. truncatula</i> )	lectin-like domains	Navarro-Gochicoa et al., 2003
3.1.1.72. Cellulose acetate esterase ( <i>N. sicca</i> SB)	CBM2	Moriyoshi et al., 2010
3.1.1.6. Acetyl esterase ( <i>A. fumigatus</i> )	CBM1	Moriyoshi et al., 2010
3.2.1.1. $\alpha$ -Amylase ( <i>L. amylovorus</i> NRRL B-4540, <i>L. plantarum</i> , <i>L. manihotivovorans</i> )	CBM26	Guillen et al., 2007
3.2.1.3. Glucoamylase ( <i>Rhizopus oryzae</i> )	CBM21: sites I, II	Chou et al., 2006
3.2.1.18. Large exo- $\alpha$ -sialidase ( <i>C. perfringens</i> )	CBM40+CBM32	Boraston et al., 2007
3.2.1.51. 1,3-1,4- $\alpha$ -L-fucosidase ( <i>B. bifidum</i> JCM1254)	CBM32+FIVAR	Ashida et al., 2009
3.2.1.-. Lacto-N-biosidase ( <i>B. bifidum</i> )	CBM32	Ashida et al., 2009
3.2.1.-. Endo- $\alpha$ -N-acetylgalactosaminidase ( <i>B. longum</i> JCM 1217)	CBM32	Ashida et al., 2009
3.4.15/24.-. Zn <sup>2+</sup> -proteases ( <i>Bacillus</i> , <i>Clostridium</i> )	Domains in B-subunit of A-B toxins	Barth et al., 2004
3.5.1.-. Peptide:N-glycanase ( <i>S. cerevisiae</i> )	Chitobiose-binding groove	Zhao et al., 2009
Peptide:N-glycanase (murine)	Mannose-binding module	Zhao et al., 2009
3.6.1.5. Apyrase ( <i>M. truncatula</i> )	(Chito) <sub>oligo</sub> -binding non-hevein type domain	Etzler et al., 1999
4.2.2.-. Chondroitin sulfate ABC lyase I ( <i>P. vulgaris</i> )	N-terminal domain	Huang et al. 2003
5.4.2.-. Phosphomannose isomerase ( <i>S. chungbukensis</i> DJ77)	Mannose-1-phosphate binding conserved motif	Tran et al., 2005
6.1.-.-. E3 ubiquitin ligase (human)	C-terminal conserved motif	Yoshida, 2007

\*Differences in occurrence of repeated combinations of domains, cooperation of sites or motifs of few aminoacids, specific combinations with another type neighbor domain, position in B-subunit. CBM: carbohydrate binding module.

Thus, probiotic bacterial lectins and related systems may serve as a power source of anti-stress activities, cytokine-like and immunomodulating factors, anti-tumor and anti-viral agents (Lakhtin et al., 2010a, Lakhtin et al., 2010b, Lakhtin et al., 2010c, Lakhtin et al., 2010d).

### 1.5 Prospects of Lectin-Enzyme Relationships

Aforementioned data indicate great importance and extended prospects of lectin-enzyme relationship studying and application in future. First of all, our knowledge on lectin-enzyme relationships will help to understand deeper mechanisms of natural processes of cell recognition and surviving under conditions of abnormal events and development of disease in organism. Indeed, many bacterial, fungal and animal lectins are the same in term of the presence of the same types of lectins (for example, C-, L-, R-, or CBM-type) (Lakhtin et al., 2010). Another example is similarity of bacterial and vertebrate F-type lectins (fucose-binding) including *Streptococcus* proteins, pentraxin-1, etc (Drickamer, 2006). Such similarly functioning lectins may reveal similar ways of lectin-directed realization of key enzyme activities at the levels of organelle, cell, tissue, organ, or biotope in human organism. In case of lectin-regulated hydrolases it may be possible to control and influence the pool of signals of different types (stress, abnormal, cross-talking, quorum sensing, hierarchic between human and microbiota, etc.). The latter aspect is of especially importance because it takes into consideration the functioning of the so called “super system of lectins in human” (components of complement, blood clotting system, protein hormone – hormone receptor system, some of defensins and cytokines, etc) (Lakhtin et al., 2009).

Secondly, aforementioned knowledge on lectin-enzyme relationships will allow development of value universal molecular and supramolecular constructions in medical and bioengineering. For example, the presence of at least 60 families of CBM in natural glycoside hydrolases (Montanier et al., 2010) will create

newly constructed CBM-containing enzymes of usefulness for probiotic, prebiotic, symbiotic and synbiotic system functioning in organism, organ, tissue and cellular models or artificial bioreactors of industrial significance. Another promise possibility may be perspective use of probiotic microbial lectins (Lakhtin et al., 2010a, Lakhtin et al., 2010b). It seems, in normal healthy conditions probiotic bifidobacterial and *lactobacillus* lectins are involved in supporting healthy level of equilibration of the system host – microbiota. Prospects of the use of human probiotic bacterial lectins can be considered as strategic direction in probiotic therapy (Lakhtin et al., 2008).

### Conclusion

At present, points of views with respect of lectin – enzyme relationships are closed, similar and converged. These relationships are universal and can be applied to any aspect of hostmicrobiota relationships. The knowledge on lectinenzyme relationships opens new promised prospects in future in construction of new generation of bioactives and drugs, biomarkers and sensors for successful use in clinical microbiology and medical biotechnology.

### References

- Abbott, D.W., Eirin-López, J.M., Boraston, A.B., 2008. Insight into Ligand Diversity and Novel Biological Roles for Family 32 Carbohydrate-Binding Modules, *Mol. Biol. Evol.* 25, 155-67.
- Aleshkin, V., Amerhanova, A., Pospelova, V., Afanasyev, S., Shenderov, B., 2008. History, present situation, and prospects of probiotic research conducted in the G.N. Gabrichevsky Institute for Epidemiology and Microbiology, *Microb. Ecol. Health Dis.* 20, 113-5.
- Ashida, H., Miyake, A., Kiyohara, M., Wada, J., Yoshida, E., Kumagai, H., Katayama, T., Yamamoto, K., 2009. *Bifidobacterium bifidum* are essential for the utilization of fucosylated milk oligosaccharides and glycoconjugates, *Glycobiology.* 19, 1010-7.
- Barth, H., Aktories, K., Popoff, M.R., Stiles, B.G., 2004. Binary bacterial toxins: biochemistry, biology, and applications of common *Clostridium* and *Bacillus* proteins, *Microb. Mol. Biol. Rev.* 68, 373-402.
- Boraston, A.B., Ficko-Blean, E., Healey, M., 2007. Carbohydrate recognition by a large sialidase toxin from *Clostridium perfringens*, *Biochemistry.* 46, 11352-60.

- Chou, W.I., Pai, T.W., Liu, S.H., Hsiung, B.K., Chang, M.D.T., 2006. The family 21 carbohydrate-binding module of glucoamylase from *Rhizopus oryzae* consists of two sites playing distinct roles in ligand binding, *Biochem J.* 396, 469–77.
- Drickamer, K., 2006. F-type lectins, [www.imperial.ac.uk/Animal\\_lectins\\_home](http://www.imperial.ac.uk/Animal_lectins_home). Enzyme Nomenclature. Recommendations 1992. Orlando: Acad. Press. 1992.
- Etzler, M.E., Kalsi, G., Ewing, N.N., Roberts, N.J., Day, R.B., Murphy, J.B., 1999. A nod factor binding lectin with apyrase activity from legume roots, *Proc. Natl Acad. Sci USA* 96, 5856-61.
- European Bioinformatics Institute 2010> Databases> InterPro.IPR008979 (Gal-binding domain-like in proteins).
- Fritz, T.A., Raman, J., Tabak, L.A., 2006. Dynamic association between the catalytic and lectin domains of human UDP-GalNAc:polypeptide  $\alpha$ -N-acetylgalactosaminyltransferase-2. *J. Biol. Chem.* 281, 8613-9.
- Guillén, D., Santiago, M., Linares, L., Pérez, R., Morlon, J., Ruiz, B., Sánchez, S., Rodríguez-Sanoja, R., 2007. Alpha-amylase starch binding domains: cooperative effects of binding to starch granules of multiple tandemly arranged domains, *Appl. Environ. Microbiol.* 73, 3833–7.
- Huang, W., Lunin, V., Li, Y., Suzuki, S., Sugiura, N., Miyazono, H., Cyglier, M., 2003. Crystal structure of *Proteus vulgaris* chondroitin sulfate ABC lyase I at 1.9 Å resolution, *J. Mol. Biol.* 328, 623-34.
- Ito, N., Phillips, S.E., Stevens, C., Ogel, Z.B., McPherson, M.J., Keen, J.N., Yadav, K.D., Knowles, P.F., 1991.
- Lakhtin, M., Alyoshkin, V., Lakhtin, V., Nesvizhsky, Y., Afanasyev, S., Pospelova, V., 2010a. [The role of lectins from probiotic microorganisms in sustaining the macroorganism (in russian)], *Vestnik of Russian Academy of Sciences (Moscow)* N2: 3-8.
- Lakhtin, M., Alyoshkin, V., Lakhtin, V., Afanasyev, S., Pozhalostina, L., Pospelova, V., 2010b. Probiotic lactobacillus and bifidobacterial lectins against *Candida albicans* and *Staphylococcus aureus* clinical strains: new class of pathogen biofilm destructors, *Probiotics and Antimicrobial Proteins* 2: 186-96, DOI: 10.1007/s12602-010-9046-3.
- Lakhtin, M., Lakhtin, V., Alyoshkin, V., Afanasyev, S., Alyoshkin, A., 2010c. [Lectins and enzymes in biology and medicine (in russian)], “Dynasty” Publishing House, Moscow, in press.
- Lakhtin, M., Lakhtin, V., Alyoshkin, V., Afanasyev, S., 2010d. Lectins of beneficial microbes: system organization, functioning, and functional superfamily, *Beneficial Microbes*, submitted to publication.
- Lakhtin, M., Lakhtin, V., Alyoshkin, V., 2011. Three lectin systems in lactobacillus consortium, *FEMS* 2011, submitted for publication.
- Lakhtin, V., 1987. [Lectins for investigation of proteins and carbohydrates (in russian)], in: A. Klyosov (Ed.), *Achievements in Science and Technique, Seria Biotechnology, Vol 2, VINITI, Moscow*, 4-290.
- Lakhtin, V., 1994. [Molecular organization of lectins (in russian)], *Molecular Biology (Moscow)* 28, 245-73.
- Lakhtin, V., Lakhtin, M., Pospelova, V., Shenderov, B., 2006a. Lactobacilli and bifidobacteria lectins as possible signal molecules regulating intra- and interpopulation bacteria-bacteria and host-bacteria relationships. Part I. Methods of bacterial lectin isolation, physicochemical characterization and some biological activity investigation, *Microb. Ecol. Health Dis.* 18, 55-60.
- Lakhtin, V., Alyoshkin, V., Lakhtin, M., Afanasyev, S., Pospelova, V., Shenderov, B., 2006b. [Lectins, adhesins and lectin-like substances of lactobacilli and bifidobacteria (in russian)], *Vestnik of Russian Academy of Sciences (Moscow)* N1: 28-34.
- Lakhtin, V., Lakhtin, M., Pospelova, V., Shenderov, B., 2007. Lectins of lactobacilli and bifidobacteria. II. Probiotic lectins of lactobacilli and bifidobacteria as possible signal molecules regulating inter- and intrapopulation relationships between bacteria and between bacteria and the host, *Microb. Ecol. Health Dis.* 19, 153-7.
- Lakhtin, V., Afanasyev, S., Alyoshkin, V., Nesvizhsky, Y., Pospelova, V., Lakhtin, M., 2008. [Strategical aspects of constructing probiotics of the future (in russian)], *Vestnik of Russian Academy of Sciences (Moscow)* N2: 33-44.
- Lakhtin, V., Afanasyev, S., Alyoshkin, V., Nesvizhsky, Y., Lakhtin, M., Shubin, V., 2009. [Classification of lectins as universal regulator molecules of biological systems (in russian)], *Vestnik of Russian Academy of Sciences (Moscow)* N3: 36-43.
- Lakhtin, V., Lakhtin, M., Alyoshkin, V., 2010. Lectins of living organisms. *Anaerobe*, in press.
- Montanier, C., Flint, J.E., Bolam, D.N., Xie, H., Liu, Z., Rogowski, A., Weiner, D., Ratnaparkhe, S., Nurizzo, D., Roberts, S.M., Turkenburg, J.P., Davies, G.J., Gilbert, H.J., 2010. Circular permutation provides an evolutionary link between two families of calcium-dependent carbohydrate binding modules, *J. Biol. Chem.*, published on July 21, 2010 as Manuscript M110.142133.
- Moriyoshi, K., Koma, D., Yamanaka, H., Ohmoto, T., Sakai, K., 2010. Functional analysis of the carbohydrate-binding module of an esterase from *Neisseria sicca* SB involved in the degradation of cellulose acetate, *Biosci. Biotechnol. Biochem.* 74(9), 100213-1-3, 2010. Doi: 10.1271/bbb.100213.
- Navarro-Gochicoa, M.T., Camut, S., Timmers, A.C.J., Niebel, A., Hervé, C., Boutet, E., Bono, J.J., Anne, I., Cullimore, J.V., 2003. Characterization of four lectin-like receptor kinases expressed in roots of *Medicago truncatula*. Structure, location, regulation of expression, and potential role in the symbiosis with *Sinorhizobium meliloti*, *Plant Physiol.* 133, 1893–1910.
- Novel thioester bond revealed by a 1.7 Å crystal structure of galactose oxidase, *Nature.* 350, 87-90.
- Sharon, N., Lis, H., 2003. *Lectins*, second edition, Kluwer Academic Publishers, Dordrecht, 450 pp.
- Shenderov, B., 2008. [Functional nutrition and its role in prophylactics of metabolic syndrome (in russian)], *DeLi print, Moscow*, 246-9.
- Tran, S.T., Le, D.T., Kim, Y.C., Shin, M., Choi, J.D., 2009. Cloning and characterization of phosphomannose

- isomerase from *Sphingomonas chungbukensis* DJ77, *Biochem. Mol. Biol. Reports* (Korean Society for Biochemistry and Molecular Biology) 42, 523-8.
- Yoshida, Y., 2007. F-box proteins that contain sugar-binding domains, *Biosci. Biotechnol. Biochem.* 71, 2623-31.
- Zhao, G., Li, G., Zhou, X., Matsuo, I., Ito, Y., Lennarz, W.J., Schindelin, H., 2007. Structural and mutational studies on the importance of oligosaccharide binding for the activity of yeast PNGase, *Glycobiology* 19, 118-25.