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METHODS OF ISOLATION OF LECTIN SUBSTANCES FROM NATURAL RAW MATERIALS

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ABSTRACT:

The most important processes that ensure the functioning of biological systems and their interaction with the outside world are specific processes of recognition of each other by molecules and cells. Lectins, interacting with glycol conjugates, are directly involved in various processes of inter - and intracellular recognition. Interest in endogenous lectins is related to their ability to activate the complement system, inactivate bacteria and viruses, and regulate the process of cell adhesion in inflammatory processes and metastasis. Galectins isolated from the kidneys, liver, and brain of animals, as well as collectins of hepatocytes, are most fully characterized.

KEYWORDS: lectins, activity, effective method, molecular weight.

INTRODUCTION:

There is little data on lectins circulating in the blood. The structure is defined only for mannan-binding lectins, serum amyloid component P, C-reactive protein and selectors, and their biological role in the human body is only partially elucidated. For all these lectins, the target cell has not been identified and a specific receptor has not been identified. Virtually nothing is known about galactose-specific serum lectins, although membrane-bound and intracellular lectins of this specificity are widely distributed. In this regard, it is of great interest to search, isolate and study the carbohydrate specificity, and subsequently - the biological function of these lectins.

To study the structure, it is necessary to work with high-purity lectins. Isolation of lectins is associated with a number of difficulties, since they are usually hydrophobic proteins, are labile, quickly lose their activity, and their concentration in the body is low.

The purpose of this work was to develop an effective method for isolation of lectins from various sources using composite sorbents based on macro porous glass with grafted polyacrylamide phase, search and isolation of lectins from human blood serum, determination of carbohydrate specificity and primary structural characteristics of the obtained proteins.

MATERIALS AND METHODS:

Scientific novelty and practical significance of the work: As a result of the research, a methodology for isolation and purification of lectins using composite sorbents based on macroporous glass with a polyacrylamide grafted phase was developed.

MS-PA – macro porous glass with polacrylaminic grafted Faim; GA • gsmagglutinating activity; RGA - hemagglutination reaction; SL - serum lectins; EF in PAAG - electrophoresis in polyacrylamide gel; GC - hydrophobic chromatography; GF - gel filtration; PBS-phosphate - salt buffer; IOC-ion exchange chromatography; HPLC - highly effective LIQUID chromatography.

To isolate the SIAL-binding bacterial lectin, two affine sorbents were synthesized, one based on MC, and the other based on a commercially activated Affi - gel matrix 102. Fetuin, a glycoprotein containing N - and O - sialated carbohydrate chains, was selected as the ligand for obtaining sorbents.

To study the more detailed specificity of SL1 and SL2, we studied the inhibition of the

hemag glutinase reaction of red blood cells by lectins using oligosaccharides containing both internal and terminal fragments of Gal - and Gal NAc, typical for human oligo saccharine chains of glycoproteins and glycolipids, including antigen determinants of atct/scoupe.

GA lectin is inhibited by low concentrations of trisaccharides H (type 1) and H (type 2), and their internal fragments Gaipi-3GlcNAcP and Gaipi-4GlcNAcP remain highly active, and the terminal H-disaccharide (Fucal-2Gal) is noticeably less active. CJI1 is also highly active against a disaccharide containing neither Gal nor GalNAc, namely, Fucal-3GlcNAcP (0.22 mm). The strongest RGA inhibitor for serum lectin CJI1 is the disaccharide Gaipi-3GalNAca in the form of 4-nitrophenylglycoside. The interaction of SL1 with the disaccharide Lec, used as a ligand for lectin isolation, is about 30 times lower than with GaipI-3GalNAca.

From the above data, it follows that two-stage affinity chromatography allows to obtain sufficiently purified CJ11 and SL2 (the degree of purification in the case of SL1 is 4140 times, in the case of SL2 - 6440 times), while the protein yield from 4 ml of blood serum is 0.028 mg and 0.018 mg, respectively (7 and 4.5 micrograms per 1 ml). A significant decrease in the Rga titer in the SL1 and SL2 fractions in comparison with the initial serum can be explained by the removal of other components of the blood serum, which, like lectins, have hemagglutinating activity ("natural" antibodies to carbohydrates) during the purification process.

Analysis of the obtained preparations SL1 and SL2 by native electrophoresis in the PAAG block showed that the molecular weight of both SL1 and SL2 is about 440 kDa.

Carbohydrate specificity of SL1 and SL2 was determined by inhibiting hemagglutinating activity. When selecting optimal conditions for the GA reaction, the possibility of using red blood cells from the blood of various animals and humans was investigated. As a result, rabbit red blood cells were selected, because they are the most accessible and allow you to get a high titer in the HA reaction.

SL1 and SL2 do not belong to either C-or S-lectins, since the introduction of EDTA at a concentration of 0.025 M and 2-mercagtoethanol at a concentration of 0.001 - 0.004 M did not affect the GA of lectins.

The maximum inhibitory activity among monosaccharides for both SL1 and CJI2 is shown by GalNAc a-and β-glycosides

In addition, the inhibition of RGA by natural glycoproteins and their oligosaccharide chains was investigated. The maximum inhibitory effect on the hemag glutinating activity of lectin SL1 is fibrinogen (0.5 mg / ml), which is 2 times more than for fetuin. Ai-Acidic glycoprotein at a concentration of I mg / ml does not have hemag glutiniru activity in relation to SL1. The pool of fibrinogen carbohydrate chains inhibits hemagglutination of SL1 red blood cells at a concentration of 0.125 mg / ml, which is 2 times higher than the concentration of inhibition of ai-acid glycoprotein and fetuin by the pool of carbohydrate chains. Apparently, SL1 recognizes the terminal disaccharide Neu5Aca2-6Gaip, which is consistent with data on determining the carbohydrate specificity of lectin using individual oligosaccharides, among which b'-sialillactose is one of the strongest inhibitors. The data obtained for SL2 looks different in the studied series noticeable ingibirutee activity is not possessed none of the glycoprotein.

Fraction 2 22 0.04 0.88 0.02 4330
Na-MS-PA
Fraction 2 20 0.03 0.6 0.02 6360
Fraction 2 rechromatography
SL1 BES-MPs b 0.06 0.36 0.01 10600

SL2 Ky-MPs 8 0.03 0.24 0.006 15900
Isoelectric focusing of SL1 and SL2
SL1 SL2 4 4 0,05 0,03 0,2 0,12 0,005 0,003 19070 38150
Monosaccharide composition of SL1 and SL2 (mole per 3 mole of mannose):
Carbohydrate carbohydrate Content SL1 SL2
Gai 2.9 2.2
Map 3.0 3.0
GlcNAc 4.0 2.7
GaINAc 1,0 0,5
Fuc 0.3 0.1
The content of Neu5Ac in SL1 and SL2 is 2%.

Comparison of the results of the analysis of the carbohydrate composition and the value of the molecular weight of the protein suggested that the carbohydrate part of SL1 is represented by one two - antenna N-and one O - glycosidic chains, and SL2 - one or two-antenna N-glycosidic chain.

According to the data of amino acid analysis, SL1 and SL2 must have strong hydrophobic properties, since they contain a significant amount of LEU, Val and Ala residues, and SL1 also has Pro. The ratio of acidic and basic amino acid residues is approximately 1:1, which is confirmed by the values of isoelectric points SL1 and SL2, determined by chromate focusing and isoelectro focusing methods in PAAG.

N-terminal amino acid sequence of SL1 and albumin from human serum: Homologous sections are highlighted.

CONCLUSION:

Despite the high homology with albumin, the difference in amino acid composition, physical and chemical properties and specificity makes it possible to consider them different proteins.

When comparing the n-terminal amino acid sequence of SL2 with the sequence of proteins from the data Bank (PIR 1995), it was shown that SL2 is close to the serum amyloid component P (SAP), the greatest homology is observed at the site 1 - 10 AK.

According to literature data, serum lectins-collectmny, C-reactive protein, serum amyloid component P, exhibit immune modulators activity in inflammatory processes. Some homology of SL1 and SL2 with acute phase proteins suggests that they belong to this group of serum proteins and may also have immune modulators activity. Therefore, further study of these lectins, including their biological function, is of great interest.

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