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METHOD FOR REMOVAL AND IDENTIFICATION OF CORONOVIRUSES FROM BLOOD BY MEANS OF LECTIN-AFFINE HEMODIALYSIS

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ANNOTATION:

The coronavirus, which has now taken over the world in a short period of time, is a virus that resembles the causative agent of atypical syphilis (SARS), a family of viruses known as TORS. This causes chronic illness. We know that as a result of this disease, many people died prematurely and the economic development of the entire world has plummeted. In such conditions, the treatment of coronavirus disease by the method of lectin-affinity hemodialysis, obtained using lectin isolated from local raw materials, is an urgent problem.

Keywords: lectin, glycoprotein, virus. Coronavirus, sorbent, hemodialysis.

INTRODUCTION:

Today there is a great demand for drugs from natural raw materials around the world. Due to natural raw materials - drugs that are developed on the basis of biologically active substances, the above animals are harmless to the human body than drugs obtained in a synthetic one. Therefore, we have set ourselves the goal of separating the lectin based on local plants.

Lectins are specifically associated with carbohydrates and are recognized as a type of sugar. The biological functions of lectin include cell interference, pathogen interactions, and the innate immune system. Lectins are mainly based on leaves, plant tissues, roots and seeds. They are glycoproteins that bind a covalent structure to special mono- or superiosaccharid. They are usually found in the seeds of some legumes. They make up 3% of the seeds of mature seeds in general. Lectin is found in living organisms, but plant lectin is the first protein.

Lectin is included in glycoproteins classes, with a place with medical and pharmaceuticals. Lectin is collected in large quantities in all legumes (including plants, soybeans, beans, peas). It is used in the purification of antibodies in the field of diagnostics in medicine, the study of mutant cells, in the purification of antes, cancer cells.

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 110 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:

It is known that lectin belongs to the class of glycoproteins, which are additional proteins that do not affect one or more carbohydrate substances and their structure. The protein-forming compounds of this carbohydrate form the basis of many important physiological processes in the body. They allow cells and microorganisms to attach to tissues, as well as maintain intercellular communication using chemoreceptors.

However, the cell membrane is transforming. These changes in the cell membrane can be studied using lectin. Due to these properties, purified lectins are widely used in a number of fields, including the purification and analysis of glycoproteins, the study of mutagenic cells, and the analysis of cell membranes.

As a result of the study, lectin was isolated from plant materials. In our next study, a lectin-affinity sorbent was synthesized, the synthesized sorbent was placed in a hemodialysis machine, and the patient's blood plasmid was passed through a porous lectin-affinity membrane. As a result, the concentration of the virus in the patient's blood drops sharply, and the patient's health gradually begins to recover.

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