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THE USE OF GLYCOCONJUGATES IN ANALYSIS OF PROTECTION AGAINST PATHOGENS: GENERAL RULES

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ABSTRACT

The overview is devoted to our own data on the use of water-soluble glycoconjugates based on a linear polyacrylamide chain in relation to recombinant therapeutic human protein hormones and probiotic recognition proteins, such as enzymes and lectins. The results obtained characterize the basic principles of multilevel relationships between proteins and glycoconjugates, including the assembly of complexes and nanoparticles on a solid phase that mimics the cell surface. The prospects of applying these principles in the study of therapeutic proteins and other protein factors and cofactors, enzymes of all classes (including carbohydrate metabolism enzymes) of microorganisms and viruses are emphasized. The presented data will help in further development of protective network communication systems of organism. The data can serve the basis in search and construction of new effective combinations of drugs against infections and pathogens. These data will support the variety of applications in medical biotechnology.

KEYWORDS: glycoconjugates; glycoproteins; carbohydrate-binding; CBM; enzyme carbohydrate sensitivity; protein factors; lectinbiotics; enzymebiotics; assemblies; recognition, communications; viruses; pathogens; probiotics.

ABBREVIATIONS

Bf : Biofilm(s)

CB : Communicative body(bodies)

CBM : Carbohydrate-binding module(s)

E : Enzymes/Enzymbiotics

Gc : Glycoconjugates

Gp : Glycoproteins

L : Lectins/Lectinbiotics

LPS : Lipopolysaccharide(s)

PAA : Polyacrylamide

PG : Peptidoglycans

PS : Polysaccharide(s)

1. INTRODUCTION

In general, glycoconjugates (Gc) include glycoproteins (Gp), glycolipids (lipopolysaccharides [LPS], others), peptidoglycans (PG), PS; a set of components of extracellular matrices and capsules, as well as intracellular origin; rare signaling or widespread natural, recombinant or synthetic glycoantigens. The recognition of Gc by proteins and/or viruses is a key step in the search of important and useful processes, as well as in study of mechanisms of binding to cells and intracellular targets. Recognition initiates degradation and lysis of pathogenic and foreign cells, destruction of microbial arrays and biofilms (Bf). Recognition of pathogens initiating infections is carried out in accordance with evolutionarily debugged mechanisms of protein-Gc interactions at different levels of the organization of the living. Such interactions involve the involvement of lectins or lectinbiotics (L), L-like and L-analog agents, as well as enzymes or enzymbiotics (E), which contribute to an increase in the pool of antimicrobial peptides in the body.

The purpose of the study is to systematize our own data for the formulation of general principles and rules (keys of use) of the relationship between L and Gc in study of protein factors and viruses, as well as for the application of these principles with the prospects of strengthening the protection of the body.

2. MATERIALS AND METHODS

Linear Gc-polymers are constructed on polyacrylamide (PAA) - a carrier that is soluble in water and aqueous solutions of salts. They include side branches of carbohydrates (25% by weight) (www.lectinity.com). Such Gc mimic polyvalent compounds, similar to "exposed mucin" with exposed clusters/sites/sites of sets of homogeneous/identical carbohydrate residues in side-mounted antennas (each antenna includes 1-3 or more residues). When interacting with Gc targets, the conformation of the "random tangle" is adapted in accordance

with the characteristics of carbohydrate-receiving sites on the solid-phase affine protein surface. Gp and other natural Gc, their assembly complexes on porous hydrophobic and hydrophilic membranes imitating the cell surface are involved in such processes. The Gc used in the work mimic natural polymer compounds of various natures (PS, PG; typical and unique, widespread glycoantigens). They can be used as models of components/partners of mutual recognition, reactions and processes in the body.

Step-by-step live image recording of chemiluminescence kinetics of interaction of electrophoretically separated and soaked proteins and L with Gc-biotin-streptavidin-peroxidase, the use of an immune sandwich labeled with peroxidase, additional fluorescence analysis of protein in the Biochemi system (UVP) in complexes and supramolecular ensembles, including during assembly, provide highly informative representations of events on a phase similar to the cell surface. The results were confirmed by measurements of antimicrobial and other biological activity associated with proteins and Gp.

In the process of establishing and developing the fundamental key principles of the relationships between L and Gc in the study of proteins, their complexes and nanoparticles, the data of our own publications were systematized.

3. RESULTS AND THEIR DISCUSSION

3.1. *Structural and functional relationships between L and Gc*

L includes "peptide/protein of non-immunoglobulin nature"-containing structures and complexes that recognize and bind carbohydrates and Gc. L have spatial 3D areas/epitopes of Gc recognition. L often function as di- and oligomeric, including in supra-subunit and supramolecular complexes. L may have Ig-like domains and epitopes co-functioning with the lectin site. L may contain enzyme (E) activities, and the site of lectin activity may not depend (be spatially separated) from the catalytic center / site of carbohydrate metabolism (the presence of carbohydrate-binding modules [CBM] in the carbohydrate metabolism site, independent of the catalytic center). Such bifunctional molecules are considered as true (not false) lectins: L-E and E-L with the mutual influence of Gc-recognizing sites (domains, epitopes, clusters, modules).

L participate in network immunity. L function as signaling factors, informosomal (including as part of phenotypic informosomes); they are auxiliary, correcting and stabilizing in relation to the dominant activities of all (sub)classes and groups of protein effectors (E, cytolysins

with a modular independent arrangement of functions/activities, hormones, cellular receptors (including enzyme nature), cytokines, defensins, antibiotics, Gc-recognizing peptides (including shortened L). Active L molecules are asymmetric, with built-up exposed or latent/masked activities. L has hydrophobic sites and co-functioning sites with lectin activity, making it susceptible to ordered/ranked Gc series. L activities often depend on Ca^{2+} cations and/or other metals. L can have 2 or more Gc-recognition sites. L reveal polyvalence, especially in supramolecular complexes, ensembles and nanoparticles, including in the contact/interfacial regions. L is characterized by the ability to direct assembly, especially in the interphase/surface areas of cells and organelles, as well as viruses.

They also exhibit functions that organize the infrastructure in biotopes, including those based on "building" bio(nano)materials (PG, layers of cell membranes and extracellular matrices, ordered mucus, organelle surfaces and cytoplasmic "skeleton") are carrier/delivery agents.

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L participate in ensuring the overall network immunity of the body. L function as signaling factors, somal (including as part of phenotypic informosomes); they are auxiliary, correcting and stabilizing in relation to the dominant activities of all subclasses and groups of protein effectors: E (including enzymbiotics), cytolysins with a modular independent arrangement of functions/activities, hormones, cellular receptors (including enzymatic nature), cytokines, defensins, antibiotics, Gc-recognizing peptides (including shortened L). Active L molecules are asymmetric (including at the subunit level), with accumulated open or hidden/masked activity/activities. L have exposed hydrophobic sites and sites with lectin activity functioning together with them, which makes L susceptible to ordered/ranked Gc sets/series. L activities often depend on Ca^{2+} cations and/or other metals. L may have 2 or more Gc recognition sites.

L exhibit and reveal polyvalence, especially in supramolecular complexes, ensembles and nanoparticles, including in contact/interfacial regions. L are characterized by the ability to assemble directly, especially in the interphase/surface areas of cells and organelles, as well as viruses.

L also exhibit the functions of organizing infrastructure in biotopes, including those based on "building" bio(nano)materials (PG, layers of cell membranes and extracellular matrices, ordered mucus, organelle surfaces and cytoplasmic "skeleton") are carriers/deliverers of the material.

L molecules are usually represented by systems of shapes (assembly, shortened; with different glycosylation, hydrophobicity and 3D landscape) with different functions and biological activities that can be modulated by external factors. L systems, in turn, are aimed at a system of Gc targets, which can be ranked according to the degree of accessibility and affinity (reversible or irreversible) to L molecules (simultaneous recognition of several different Gc targets separated in space is possible). L systems are mosaic (for example, in the process of visualizing separated forms of L manifested by one type of Gc); they are characterized by synergism (for example, with non-competitive systems from the same source) when achieving the final result, including antimicrobial and antiviral in combination with other antimicrobial drugs.

L are able to assemble into (nano/micro) particles, latex-type particles and filaments - varying multivalent forms of L (macro-L) that recognize Gc, which are also formed in the case of cell sensitization with L (particles and cells as model macro-L, suspension particles [suspension forms of macro-L] and cell forms of macro-L). Macro-L can exhibit lytic, destructive and regulating/modulating effects in relation to microbial arrays - microbiocenoses in biotopes and as part of a single symbiotic/probiotic compartment of the organism.

3.2. *General rules as a current keys for consideration of the relationships between L and Gc*

We obtained experimental data and based on them formulated the principles (the most general, primary) interactions of L and Gc corresponding to events in the human body. Some suggestions and conclusions have been made about the relationship between proteins/enzymes /L and Gc of the most general type[1-6], with respect to therapeutic proteins[7-19], E[3, 20-23] and probiotic L[24-58], as well as with respect to all factors together or

taking into account other human protection systems against pathogens that do not contain antibodies.[59-74]

Proposals on the regularities of interactions involving synthetic polymer analogues of natural Gc, natural Gc themselves, combinations of L and Gp in affine reactions in solutions and on solid changing surfaces have been used and are listed below. The manifestations of multiple weak L-Gc-affine interactions are noted in the following aspects, can be applied in studies of protein factors and viruses (the potential of application is in parentheses):

*Synergy of Gc action:
-visual, positional, mosaic, in time, combined; in Gc reactions that stained, "finished" the assembly of the polymer complex /nanoparticles in the solid phase (viruses such as macro-L and/or macro-Gc):
-- synergy of Gc with each other within and from the point of view of designated localized Gc systems, in image comparison reactions/patterns of separated multiple forms of therapeutic protein or Gp in the solid phase, "colored" by each of the compared types of Gc (variants of the predominant action of viruses as macro-Gc with the resulting choice of desired cases);
- synergism of Gc with antibodies, including monoclonal, in the process of directed phased solid-phase assembly into a "sandwich"/multi-sandwich and multi-molecular ensemble (synergism of the phage as macro-Gc or macro-L with antibodies);
- synergy between surfaces/areas with different Gc mosaics, on the one hand, and proteins/Gp/L, on the other hand (between viruses, as between interconnected systems macro-Gc and Macro-L; in the processes of initiation of assembly and crystallization of viruses);

*the ability of synergistic Gc sets to recognize and bind protein factors and cytokines in the compositions of complexes, nano- and microparticles (synergism in terms of the effectiveness of modulation of the selected activity/combined activities of the virus);
*the ability of the Gc set to sequentially/stepwise/cascade bind to various sites of multivalent nanoparticles in L-dependent assembly processes (viruses in the form of L-dependent assembly nanoparticles):
-diagnostics of L forms with maximum and minimum investigated activity (ranking of types of preferential activity) associated with the involvement of type or types of Gc;

-maximum visualization of L forms specific to the tested Gc, including synthetic analogues, in a characteristic assembly package (viral automated cell recognition nodes associated with the penetration of phage blocks; nodes as assembly, for further assembly):
-- prognostically expected assembly on a hydrophobic porous soft/membrane carrier;
-- prognostically expected assembly of affine probiotic-sensitized L and/or Gc human cells in a micropanel on a hydrophobic solid material - polystyrene;
-to display the features of the subunit, molecular and supramolecular structure in assemblies (in the components of the machine nodes of the virus);

*ability of 1-2 types (or more) Gc selected from a series of pre-selected for testing in reactions with Gp/protein factors and therapeutic agents:
- distinguish effectors with preserved selective activity in molecular complexes, supramolecular ensembles, nano/micro-functioning particles, particles with preset/programmed functions; receptors and receptor-like assembly structures on the cell surface and Bf (including in Bf pores); compositions with oriented recognition molecules such as L and Gc of other/additional types (variants of effector viruses, depending on affinity to Gc);

*the presence of Gc in the spatial field as a system when at least one parameter of Gc component coordination is known (coordination of a set of viruses as a set of Gc macroforms, including in phage cocktails):
- main and secondary forms as systems and subsystems: ordered, mosaic, multiple forms of Gp with proximity to Gc, including in the form of complexes, with unequal intensity of activity modulation;
-asymmetry with pronounced generalized vectorality of the Gc system;

*representations of cells, nano/microparticles Gp, Gp-Gc, Gc-Gc as multivalent/multifunctional/dispatcher macroforms of recognition and binding of predominant Gc types (viruses as effector forms with directed action of Gc types):
-testing of L and/or Gc in a micropanel with respect to color cellular reactions, for example, hemagglutination, in variants of modulation (additional stimulation) of cellular reactions as a result of adhesion of L/Gc in lower concentrations compared to titers when determining L/Gc using sensitized cells (testing of additional types of Gc targets in modulation reactions by proteins and viruses);

*preservation of polymer effectors (Gp, Gc and their complexes and ensembles) as part of functionally active complexes and nanoparticles with Gc, the possibility of further sequential selective cascade binding of various/non-repeating Gc types in a cascade (using two or more types of signaling Gc ordered in cascade mode when modulating/switching the action of viruses);

*identification of the first and detection of new, including expanded modified first, Gc target recognition sites under conditions of sequential assemblies of protein and Gp subunits/molecules into supramolecular complexes, ensembles and particles (regulation in viruses of the resulting basic specificity of particles, their nodes, regions and fragments to Gc of the desired type and sets of desired Gc as promising signals):

- expansion of the potential of Gc recognition by therapeutic protein/Gp (approaches to solving problems of modulation and control of viral activity; acquisition of new activities by viruses, including in associates; partial degradation or directed aggregation of viruses, including during storage, aging, in conditions of selective phases of the life cycle):
-- an ability to form new recognition sites in contact compounds in complexes, supramolecular ensembles and protein-based particles;

- the ability of Gp to recognize the presence and distribution of exposed cluster and individual types of glycan antennas in space - the sensitivity of mapping to the nano-Gc mosaic (signaling Gp and their [glyco]peptides in modulating the activity of signaling sites of the 3D map of the virus):

-- sensitivity of navigation/signal of Gp-containing target complexes and nanoparticles to the topography of the exposed targets:
--- clusters/ repeats/ "extended sites" for mosaic antennas of natural or synthetic glycans exposed in Gc (when managing virus systems and individual virus representatives);
--- Gc-mosaics as "dots on a 2D map"/spots on the expanded porous hydrophobic/hydrophilic surface of membranes and other (nano) (bio) materials and structures (preparation of affine copies for targeted communications involving viruses);
--- pronounced 3D-phenotypic informational neoplasms in contacts between Gp, Gp/Gc, Gc/Gc - such as folds, depressions, gaps, cracks, cracks, fractures, and other damages (establishment and standardization of the features of characteristic/specific/directed aggregation of viruses, including in the tail region of phages [related to the issues of virus

crystallization], as well as the impact on the viability, aging and cycle of viruses; 3D-modulation of recognition and other functions of viruses – L-like and E-manifested);

* visual template correspondence between Gc systems and "Gc recognition Proteins", systems where "One Gc acts in the direction of a set of proteins/L", "One protein/L is directed to a set of Gc"; the presence of a correspondence between protein/L and Gc within subsystems; establishing ranking orders with the identification of Gc and protein/L subsystems, depending on the implementation of activities (building a network of such relationships, taking into account the concepts of the virus as a megapattern of Gc and L sites, as the dominant type of macrogen [combinations of genes, including functionally linked] or the dominant type/types of macro-L/macro-E):
- one Gc-type can simultaneously interact with several L/(sub)L mosaic systems, which can be ranked according to proximity and accessibility to the Gc type;
- one L-type can simultaneously interact with several different Gc/(sub)Gc mosaic systems, which can be ranked according to the degree of proximity to type L;

* prediction of the potential of Gc-immunomodulatory/prebiotic/antimicrobial/antiviral action, for example, in fucan analogues, other neo-PS and neo-PG - to support biotope pro/synbiotic microbiocenoses and compartments, including those that increase the survival of local, symbiotic and probiotic lactobacilli, bifidobacteria, as well as mixtures of bifidobacteria with lactobacilli, initially isolated from the intestines of a healthy person:

- using a modulating testing method, for example, prebiotic stimulation, growth of arrays and Bf of bifidobacteria on heparinized surfaces with metal cations in the presence of a synthetic polymer analogue of Gc (using phages as macroforms of Gc- or L-suspension macroforms/nanoparticle forms of predominant Gc instead of molecular and supramolecular variants of Gc or L):

-- development of optimally effective affine bio(nano)materials compatible with biological fluids and biotopes, as well as conditions for the realization of the desired activity of synthetic Gc:

--- increasing the survival rate of pro/post/synbiotic strains and consortia intended for delivery to the human body;

--- testing of biocompatibility of combinations of effectors of fundamentally different nature (proteins, antibiotics, salts, factors, cofactors, sensitized affinity materials) with respect to the effects on film microbiocenoses and CB;
--- establishment of an effective multi-stage efficiency in a (sub)network of reactions simulating a single defense of the body;

*the presence of Gc in the spatial field as a system when at least one parameter of Gc component coordination is known (coordination of a set of viruses as a set of Gc macroforms, including in phage cocktails):
- main and secondary forms as systems and subsystems: ordered, mosaic, multiple forms of Gp with proximity to Gc, including in the form of complexes, with unequal intensity of activity modulation;
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*identification of the first and detection of new, including expanded modified first, Gc target recognition sites under conditions of sequential assemblies of protein and Gp subunits/molecules into supramolecular complexes, ensembles and particles (regulation in viruses of the resulting basic specificity of particles, their nodes, regions and fragments to Gc of the desired type and sets of desired Gc as promising signals):

- expansion of the potential of Gc recognition by therapeutic protein/Gp (approaches to solving problems of modulation and control of viral activity; acquisition of new activities by viruses, including in associates; partial degradation or directed aggregation of viruses, including during storage, aging, in conditions of selective phases of the life cycle):
-- an ability to form new recognition sites in contact compounds in complexes, supramolecular ensembles and protein-based particles;

- establishing the adequacy of Gc interactions involving L and E, causing irreversible destruction and lysis of opsonized/labeled pathogenic bacteria, as well as containing pathogenic bacteria Bf (establishing similar patterns when using phages);

* organized tactical attack involving pro/synbiotic mosaic (for example, on disks) against pathogenic CB and Bf in areas with increased sensitivity or increased resistance of CB and Bf to destruction by effectors [peripheral/borderline and central/internal regions of CB, respectively] (phages instead of a point/disk antibiotic in the central or peripheral area of a hydrophilic porous gel plate with target bacteria, to determine biocompatibility and synergism of phages with L and Gc):
- achieving the state of "multicenter CB" with the help of time/kinetic sequential characteristic patterns;
-modeling 2D [taking into account disks] / 3D [taking into account holes, depressions, lacunae] - probiotic functionally related microbiocenosis and probiotic biotope compartment (also taking into account phage cocktail as another/complementary component of probiotic biocenosis of hydrophilic porous 3D gels);
- establishment of a residual diagnostic and prognostic predominantly localized center of CB pathogenicity in a pro/synbiotic environment and/or under conditions of a pro/synbiotic attack in a model biotope of interest in the form of a series of predicted expected visual patterns;

* violation of the assembly of Bf on probiotic drugs (including those that are Gc and/or are gradually associated with Gc) may lead to increased Bf lysis (possible modulation of degradation and lysis of Bf during the assembly of Bf on phage types or phage cocktails, including after preliminary analysis of "weakened"/vulnerable sites in pathogenic CB, including using Gc mapping/CB mapping).

4. CONCLUSION

The above data indicate the relevance and prospects of ideas and principles concerning macro/mega/micro/nano/molecular interactions within 2D/3D ordered partner systems of the mosaic space between Gc and L. The rules and principles can be applied for a more complete assessment of the features of receptor-recognizing interactions of viruses and microorganisms in complex systems, including in the Phage (Phage Cocktail)—Bacterium (Bacterial Consortium) system, which is important for improving the reliability of biocontrol, as well as in the development of therapeutically significant proteins, viruses, virus-based structures, microorganisms.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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