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REVIEW

Carbochips: a New Energy for Old Biobuilders

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Microarray technology has come of age for use in high-throughput operations and large-scale studies. It allows rapid and simultaneous detection of thousands of parameters within a single experiment. Recent developments in the field of carbohydrate microarray technology facilitate applications for different types of protein–carbohydrate interactions. These developments included capture molecule immobilization, surface engineering and detection strategies to analyze entire glycomes and glycosylation in vertebrate systems, the most common post-translational modification.

[**Key words:** carbochip, glycoprotein, surface engineering, glycoproteomics, carbohydrate microarrays]

In the new scientific arena, the unscheduled and uncontrolled behavior of tissue cell societies are paving ways for the development of various research tools. To investigate the intricacies of life, it is important to resolve the mysteries of the ultimate molecular machine: the cell. To fulfill this goal, the "omics" journey, which started with genomics, has come all the way to functional proteomics (Fig. 1).

Glycoproteomics, which was a relatively inactive field in the scientific world a couple of years ago, has now become a major academic and clinical research priority. The microarray, a powerful tool in this omics era providing new opportunities to researchers, has been the major impetus behind this remarkable transformation. There is a brain-teasing structural diversity to glycans and they are bound to proteins in a variety of complex ways (1). The glycans are subjected to regulation by more complex structural guidelines (branching events, stereoisomerism and linkage forms) than DNA or proteins. For a given reducing hexasaccharide, the number of possible structural isomers is 1.05×10^{12}, for a hexapeptide, 4096 and for a hexanucleotide, 6.4×10^{7} (2). In order to drive the molecular engine of various processes (Fig. 2), protein–carbohydrate interactions provide fuel. A recent example of new microarray technology, the carbochip, provided a much better solution for the systematic and high-throughput elucidation of this specific kind of interaction. This new powerful tool has opened the gateway to a knowledge bank which was closed until now. Glycobiology is an umbrella term covering a plethora of areas. According to Dale Cumming of GlycoDesign Inc. (Canada), it is "a bit like the proverbial onion where one goes through one layer and discovers that there are whole new layers of complexity to be addressed". The integration of various mass spectrometry-founded approaches to carbochip technologies has illuminated the dark corners of glycobiology.

This review aims to summarize recent progress in the area of carbochip technology including capture molecule immobilization, surface engineering, detection strategies, and applications to analyze various protein–carbohydrate interactions.

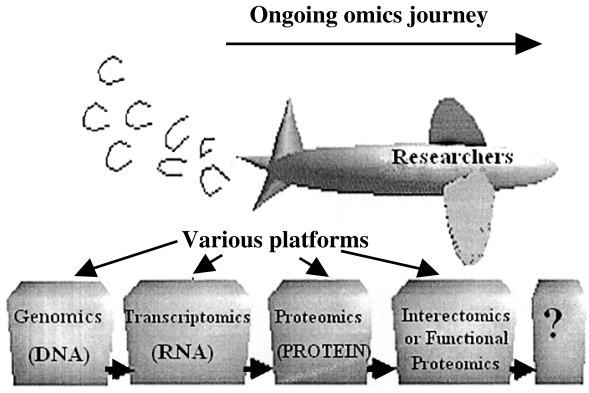

FIG. 1. Ongoing omics journey: various platforms. Representation of the recent ongoing progress of omics technologies. Progressive investigation of new research tools unraveling the dynamic knowledge of life.

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Abbreviations: BODIPY, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid; CFG, consortium for functional glycomics; DC, dendritic cell; DC-SIGN, DC-specific ICAM-3 grabbing nonintegrin; FITC, fluorescein isothiocyanate; FucT, fucosyltransferase; HRP, horseradish peroxidase; PHA-I, *Phaseolus* agglutinin I.

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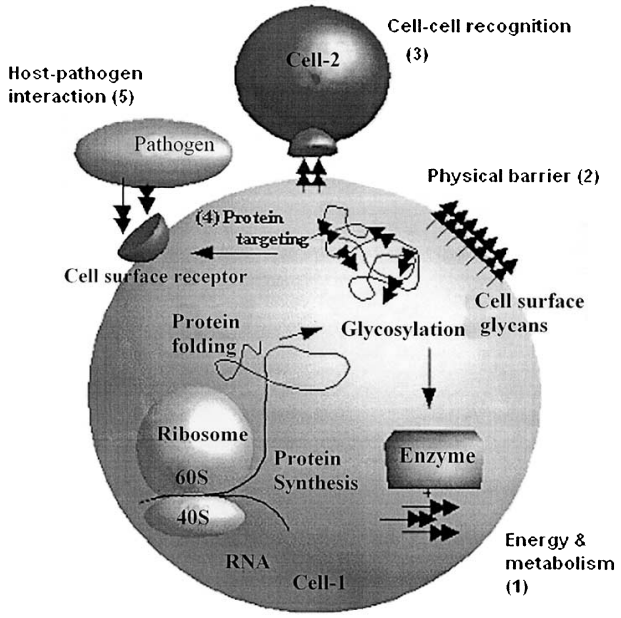

FIG. 2. One fuel for several molecular engines. Schematic representation of various biological processes governed by carbohydrate molecules: 1, energy and metabolism. Perplexities of the sugar structure morphology reveal how sugars store energy. Carbohydrate-modifying enzymes generate energy and synthesize large molecular weight storage molecules (*e.g.*, glycogen). 2, Physical barriers. Considered as weapons against external physical stresses (*e.g.*, freezing) and biochemical attacks (*e.g.*, proteases) (29). 3, Cell–cell recognition. Modulates various processes such as adhesion, activation, migration and cell to cell signaling which bears fruit in terms of fertilization, development, differentiation, transformation, apoptosis and immune response (*e.g.*, wounded tissue stimulates endothelial cells to express selectins for the recruitment of white blood cells). 4, Protein integrity. Involved in protein folding, 3D structure maintenance and trafficking of proteins (*e.g.*, glycosylation assists by forming a tag for protein targeting). 5, Host-pathogen interaction. Pathogenic aliens mimic various glycans which are usually located on the outer surface of cellular communities and thus they take advantage of this to cause infection and pathogenesis (*e.g.*, *Helicobactor pylori*).

I. WHY ANOTHER CHIP?

A microarray can be considered as a collection of miniaturized chemical reaction areas that may also be used to detect proteins or polynucleotide fragments, but carbochips have a more intricate framework than other microarray technologies registered for global glycoproteome cataloging. There is no direct link between gene expression and glycan endproducts. The structure and composition of glycans are guided by sophisticated batteries of biosynthetic enzymes operating principally within the endoplasmic reticulum (ER), the Golgi complex or the plasma membrane and this is the reason why unveiling the enigma of carbohydrate biobuilders is an extremely daunting task. The development of this new search engine has unmasked various pieces of information, which were not previously easily obtainable. Currently, one can investigate various carbohydrate interactions for their use for medical purposes (Table 1).

II. CARBOCHIP ERA TO DATE

Every chip-based experiment involves two components viz., an immobilization surface and the biomolecules to be immobilized. In general for carbochip technology, microspots of saccharide molecules are printed in rows and columns onto a solid support and further displayed to the solutions containing corresponding interacting partners. Interpretation systems are mainly based on fluorescence, radioactivity, chemiluminescence or mass spectrometry. At this juncture, various research groups have followed different technical strategies, and the excitement of innovations and discoveries fuels efforts to develop new and more advanced carbochips. The generally followed characteristics are as mentioned in Table 2.

III. EFFORTS TO DEVELOP THE MOST EFFECTIVE CARBOCHIP

Although the latest advent in carbochip technology is expected to change the pace and scope of biological research, it is still in its infancy. A schematic overview of carbochip technologies is depicted in Fig. 3. In order to be availed of by the broader research community as a future molecular biology tool kit, it requires further development. The present situation is similar to standing in front of the tree of glycoknowledge and only being able to pluck the fruits from lower branches. To be able to reach out to the top branches, one needs a taller and stronger ladder of carbochips. The ancient biobuilder, glycan, continues to pose a bigger challenge than DNA or proteins. It does not follow the standard molecular protocol of central dogma (DNA $\rightarrow$ mRNA $\rightarrow$ protein) and this is the reason why well-documented and user-friendly molecular biology techniques like cloning, sequencing, PCR amplification, and expression strategies cannot be employed with these molecules. The authors are engaged in the study of glycosylation of the enzymes of carbohydrate metabolism (unpublished work).

Carbohydrate microarray technology is plagued by various shortcomings and technical challenges, which are being sequentially discussed in conjunction with various aspects such as sample preparation, surface chemistry, instrumentation, data handling and management.

Large carbohydrate library For broad range characterization and to realise the full potential of this new tool, it is crucial to establish a high-quality storehouse of carbohydrate biobuilders. However, generating a large-scale combinatorial carbohydrate library is a very difficult task, associated with a variety of problematic issues. Using conventional chromatographic techniques such as ion exchange chromatography, the purification of stereoisomers is difficult and blood-derived samples are usually associated with problems such as rapid degradation and erythrocyte agglutination. Since the efficiency of immobilization on a surface is directly proportional to the molecular mass of the biomolecules, only oligosaccaride and polysaccharide preparations can be used. The first carbochip approach by Wang *et al*. (3) was much more feasible for polysaccharide preparations, which were covered by nitrocellulose-coated glass slides. Using the same approach, Wang *et al*. (4) raised the

TABLE 1. Applications of carbochips

Application	Description
Enzyme activity profiling	It is generally used in substrate-affinity mapping of various sugar-modifying enzymes (*e.g.*, glycosyltransferases, kinases and glycosidases) and characterizing the specificities of enzymes.
Quantitative inhibition assay	Screening of low molecular weight inhibitors of protein–sugar or sugar–sugar interactions. Main goal in the field of drug discovery is identifying glycomimetic non-natural sugars in order to augment their low bioavailability unlike their natural counterparts. The knowledge obtained can be used for designing novel drugs.
Post-translational modification	High-throughput and schematic detection of post-translational modifications viz., glycosylation and glycation.
Expression pattern analysis	Determining cell or tissue-specific expression patterns and identification of temporal expression patterns of carbohydrate residues during the process of maturation and development (*e.g.*, cancer cells change their glycosylation pattern to invade other areas).
Microbial diagnosis	Simultaneous detection of a wide range of microbial infections using limited quantities of clinical specimens by glycome analysis. This knowledge can be further used in the fight against bioterrorism.
Biomarker validation	In pharmacoglycomics, carbochips can be used for the segmentation of patient populations and for searching biomarkers for personalized medicine.
Discovery of novel carbohydrate-binding proteins	To identify the novel proteins that contain carbohydrate-binding domains. The sequence data of these proteins can be used in evolutionary studies.
Target discovery	Screening of combinatorial peptide libraries (*e.g.*, phage display library). Lectins can be regarded as protein interpreters of the sugar code (25). Carbochips are commonly used for profiling of lectin binding specificities.
Epitope mapping	Profiling of broad spectrum antibody specificities.

issue of the involvement of autoimmune responses in the pathogenesis of severe acute respiratory syndrome (SARS).

In the neoglycolipid technology applied by Fukui *et al.* (5), lipid isolation and manipulation is extremely difficult. To overcome these problems, the laboratory synthesis of sugars was attempted. However, it is currently impractical to synthesize the full complement of known sequences in animals such as *O*- and *N*-glycans of glycoproteins, diverse sequences of glycolipids and glycosaminoglycans (6). Terminal saccharide units are initially recognized by proteins, but it is very difficult to maintain the closed ring structure and the anomericity (*e.g.*, α and β anomeric configurations) of terminal saccharide units during chemical synthesis. Mizuno *et al.* (7) postulated that immobilization of the glycosyl amino acids allowed the preparation of glycochips that maintained the whole structure of the oligosaccharide. There are branched chain structures in many carbohydrates. The cluster effect (mass affinity due to multivalency) is another significant issue during solid-phase synthesis. Simultaneous multiple selective protection, deprotection and maintaining the blocking efficiency at each step is a prerequisite for the multivalent morphology of sugar structures. This mass affinity is the major obstruction for correct spacing, correct orientation and uniform densities of the fabricated sugar frameworks. It can be considered as a huge barrier to the regular and homogeneous microenvironment that results in lower biological activity and low solvent accessibility of the arrayed samples. High cost and the limited availability of enzymes (*e.g.*, galactosyltransferase) is commonly associated with the enzymatic synthesis of glycan molecules and until now no one has developed a highly efficient genetically modified biological system for the production of carbohydrate molecules. Fazio *et al.* (8) implemented the cycloaddition reactions between azides and alkynes. They used carbohydrate arrays to study known inhibitors of the fucosyltransferase (FucT) enzyme, whereas Bryan *et al.* (9) used the same array technique for the screening of a library of possible FucT inhibitors.

The carbohydrate synthesis/protein expression core of the consortium for functional glycomics (CFG) recently generated a tool box of over 75 compounds covering a broad range of structures from simple monosaccharides to decasaccharides. Clumsy nomenclature and difficult structure predictions also impede the characterization of these molecules. For structural analysis, chromatography and mass spectrometry are more often used in combination. The analytical glycotechnology core of the CFG is focusing on the development and application of ultra-high sensitivity mass spectrometric strategies for the characterization of glycoproteins, which will also allow the identification of isomers that have the same molecular weight but different structures. To avoid the confusion of nomenclature, Glycominds generated a syntax that describes the branched structures of glycans as linear computer-friendly mathematical formulae (10).

Surface engineering and instrumentation Direct and fixed stabilization of diverse glycans onto a slide surface is the essential element of all carbochip-based applications. It is also necessary for the identification of appropriate functional groups, which can promote permanent and quantitative immobilization of target molecules in defined orientations. The linker molecule hypothesis initially proposed by Park and Shin (11) can be readily adapted for diverse categories of substances such as silicon, glass, plastic, metal, fiber optics, filter membranes and so on. Linkers of suitable size make use of whole-cell glycan for correct interaction with the species to be detected and are also helpful in avoiding nonspecific adsorption. The approach proposed by Houseman and Mrksich (12) has significantly reduced the background noise and is principally based on a mixture of two alkanethiols. Ratner *et al.* (13) also observed very high signal-to-noise ratios in their study. The conjugation strategy

TABLE 2. Technical aspects of carbohydrate microarrays

Spotted molecules	Surface chemistry and engineering of spotted molecules	Molecules/Cells detected	Reference
Dextran, inulin, bacterial polysaccharides etc.	Noncovalent spots on the nitrocellulose-coated glass slides	Biotinylated, fluorescein isothiocyanate (FITC)-labeled antibodies, streptavidin-Cy3 and alkaline phosphatase-conjugated secondary antibodies	3
Cyclopentadiene-conjugated monosaccharides	Gold-coated glass slides converted into self-assembled monolayer surfaces by pretreatment with a mixture of two alkanethiols (inert ethylene glycol and hydroquinone) and sugar conjugates immobilized by the Dields–Alder cycloaddition reaction	Five rhodamine-labeled plant lectins (concanavalin A, *Benderia simplicifolia* I, *Erythrinia cristalli* lectin, *Ulex europaeus* I and *Galanthus nivalis* lectin)	12, 26
N-Acetyl glucosamine, lactose, maltose, cellobiose etc. converted into glycosylamines and coupled with maleimide linkers	Maleimide-conjugates immobilized by Hetero-michael addition on thiol-containing modified glass slides	Three FITC-labeled plant lectins (concanavalin A, *Erythrina cristagalli* lectin and *Triticum vulgaris* lectin)	11
Glycoprotein, proteoglycan, polysaccharides, glycolipids, and whole organ preparations converted into neoglycolipids by reductive amination with aminolipids[a]	Noncovalent spots on nitrocellulose or poly vinylidene fluoride (PVDF) membranes	Monoclonal antibodies (mAbs), E and L selectins, chemokines (RANTES[b]), cytokines (interferon-γ)[c]	5
Mono-and oligosaccharides conjugated with hydrocarbon chains (under stereochemically controlled conditions)	Sugar allyl derivatives noncovalently spotted on microtiter plate	Three phosphatase conjugated anti-lectins, mAb or lectins (concanavalin A, ricin B chain, *Tetragonolobus purpureas* lectin	27
Mono-and oligosaccharides converted into azide forms	Long aliphatic hydrocarbon chains (14C) noncovalently immobilized on microtiter plates and azide derivatives added by 1,3 dipolar cycloaddition reactions (*in situ*)	Alkaline phosphatase-conjugated anti-lectin mAb or fluorescence-labeled lectins (ricin B chain, *T. purpureas* I, *Sambucus nigra* lectin)	8, 28
Bacterial polysaccharides	Linker noncovalently coupled to the microtiter plate	Antiglycan and anticellulose antibodies, various biotinylated lectins (*e.g.*, wheat germ agglutinin, concanavalin A) detected by europium-labeled streptavidin	17
Biotinylated oligosaccharides	Streptavidin-coated plates (that contained monosaccharides, disaccharides, trisaccharides and larger structures, including both neutral and acidic sugars containing either sialic acids or sulfate)	FITC-or ^{125}I-labeled wild-type and mutant DC-SIGN and DC-SIGN related	15
Monosaccharides and oligosaccharides	Carbohydrates were covalently coupled via a flexible linker to the 96-well ELISA plate (microtiter plate)	DC-SIGN and Horseradish peroxidase (HRP)-labeled secondary antibodies	18
Fmoc-glycosylasparagines	Fmoc-glycosylasparagines immobilized on 96-well plates (microtiter plate)	HRP-labeled lectins concanavalin A, *Ricinus communis* agglutinin 120, *Datura stramonium* agglutinin, wheat germ agglutinin	7
Monosaccharides and oligosaccharides	*p*-Aminophenyl glycosides were covalently attached to the glass surfaces in wells via an oligomeric 1,8-diamino-3,6-dioxaoctane linker (17)	Fluorescence-labeled intact cells, chicken hepatocytes, human CD4+ T-cells	19
N-Acetyl lactosamine (LacNAc)	LacNAc was noncovalently displayed on the surface of microtiter plates via Cu(I)-catalyzed 1,3-dipolar cycloaddition with lipid alkynes	FucT, fucose-specific lectin from *T. purpureas* conjugated to a peroxidase.	9
Mannose and galactose	The monosaccharides were treated with a thiol-containing ethylene dioxy linker at the anomeric center and coupled to maleimide activated BSA and then attached to glass microspheres (with internally entrapped fluorescent dye BODIPY) using water-soluble carbodiimide	Fluorescence-labeled concanavalin A and cyanovirin N from *Nostoc ellipsosporum*	14

Spotted molecules	Surface chemistry and engineering of spotted molecules	Molecules/Cells detected	Reference
Mannose, galactose, high mannose oligosaccharides like linear trimannoside, hexamannoside and branched trimannoside	Synthetic oligosaccharide structures immobilized covalently on maleimide-activated BSA-coated glass slides by way of a hydrophilic thiol linker, whereas the remaining maleimide groups on the surface were subsequently blocked with 3-mercaptoproprionic acid	FITC-labeled concavalin A BODIPY-labeled cyanovirin N	13
Sulfhydryl-containing ethylene glycol-derivatized natural and modified glycoproteins as well as neoglycoproteins	Prepared samples fabricated by reacting amine-modified glass slides with ethylene-glycol disuccinimide to form a hydrophilic, amine-reactive surface. Slides were subsequently quenched in a solution of BSA to inactivate remaining succinimidyl groups	Fluorophore-labeled proteins DC-SIGN, antibody 2G12, cyanovirin N, scytovirin, soluble CD4	21
Collection of 51 carbohydrate antigens including both microbial polysaccharides and cellular glycan complex carbohydrates	Noncovalent spots on the nitrocellulose-coated glass slides	Horse antisera containing anti SARS coronavirus antibodies and anti *Streptococcus pneumoniae* type 18 polysaccharide antibodies, anti asialoorosomucoid (ASOR) IgG antibodies, lectin PHA-L/avidin FITC, lectin *Griffonia simplicifolia*-1-B4/avidin FITC	4

[a] Neoglycolipids (NGL) technology was employed for sample preparation.
[b] RANTES, Regulated on activation normal T-expressed and secreted.
[c] Ligand-positive components were further determined by a deconvolution strategy (which includes TLC and mass spectrometry) (see Fig. 3).

followed by Adams *et al*. (14) achieved adequate spacing between the underlying polymer support and the attached carbohydrate molecules. It also resulted in maintenance of the concentration of immobilized carbohydrate probes. Guo *et al*. (15) also followed the same strategy and demonstrated that dendritic cell-specific intercellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin (DC-SIGN) and DC-SIGN-related have distinct ligand-binding properties.

With the evolution of technologies, more sensitive, accurate, cost-effective and rapid automated equipment may be available in future. The array will be highly reproducible (plate to plate, well to well and batch to batch reproducibility), stable and it will be possible to dry-store it for several months. Novel surface slides generated by Willats *et al*. (16) were stored for three months at room temperature. The array protocol conducted by Schwarz *et al*. (17) was much more reproducible. Su *et al*. (18) also followed the same strategy and demonstrated the presence of an alpha-anomeric glycosidic linkage contributing to the high-affinity binding of cognate oligosaccharide ligands. Nimrichter *et al*. (19) followed the same strategy and extended the applicability of glycan microarrays to detect and quantify the specific adhesion of intact cells to covalent carbohydrate microarrays engineered on glass slides. By this methodology, carbohydrate specificity could be screened on primary cells without purifying and expressing the relevant lectin(s). This indicates that both protein–carbohydrate and carbohydrate–carbohydrate interactions are amenable to cell-based microarray studies. Up-to-date fabrication strategies and modern printing methods will need to be developed very soon because it is a major shortcoming to maintain the printing load (higher printing load rapidly degrades the saccharide molecules). It is also strategic to formulate suitable software for data normalization. For the detection strategies, the critical point is how precisely weak interactions can be determined and this is the major reason why current techniques are giving low quantitative performances. Lectin–sugar interactions are relatively weaker when compared with antigen–antibody interactions (20). Adams *et al*. (21) demonstrated the potential of carbohydrate microarray analysis by probing the carbohydrate affinity and structural requirements of four well-known and relevant glycoprotein 120 (gp120) binding proteins, and scytovirin, a novel HIV-inactivating protein. Such studies provide vital information for designing vaccines that give immune responses to HIV infection.

IV. COMPUTATIONAL BIOLOGY

In the foreseeable future, a huge data explosion can be anticipated as a result of the recent developments. Thus, it is vital to develop a standard protocol for data mining, data handling and management for convenient computational analysis. The Human Protein Reference Database (www.hprd.org) has the potential to fulfill this requirement. This database contains 226 glycosylation sites among the 7168 human proteins annotated as on the date of communication. Moreover, the agreement between glycominds and CFG has been strengthened with the incorporation of 1500 structures into the Carbohydrate Database.

Although carbohydrate microarray technology is still not comprehensive and major challenges exist even now for large-scale studies, with the aid of mass spectrometric (MS)-based approaches researchers can now perform high-throughput operations for glycan and protein glycoform cataloging. The identification of glycosylated peptides is generally carried out by matrix-assisted laser desorption/ionization (MALDI)-MS or LC/MS fingerprint analysis, whereas the location of modified amino acids can be obtained from fragmentation spectra generated by nano-electrospray ionization/MS/MS or LC-MS/MS analysis. For more comprehensive characterization of glycoproteins, direct MS-based approaches, such as glyco-catching can be applied (22). In

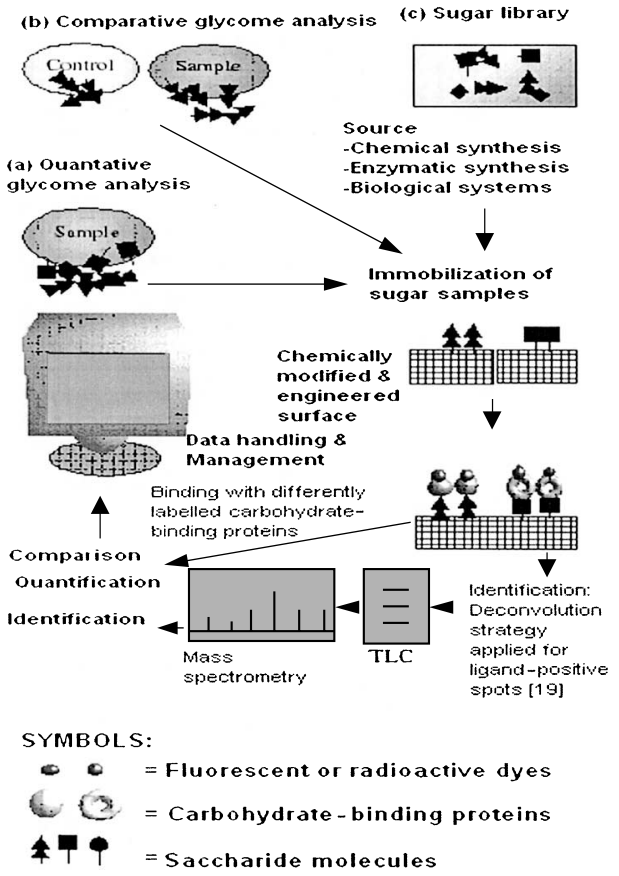

FIG. 3. Overview of carbochip technology. Schematic representation of carbohydrate microarray technology used in various experimental protocols for sugar analysis: (a) Quantification of saccharides. Single-source isolates containing different saccharide molecules can be quantified in parallel. Immobilized samples are further detected by labeled detection species. Comparative analysis with the control spots (positive and negative) and calibration spots produce accurate signal quantification. (b) Comparative analysis of saccharides. In the realm of pathological and morphological studies, different isolates for particular saccharides can be compared with the help of labeled binding partners. These primary arrays are further subjected to cross-matched analysis for comparison. (c) In neoglycolipid technology, deconvolution strategies with TLC and mass spectrometry have been applied for the assignment of carbohydrate sequences recognized by carbohydrate-binding proteins (CBP). This strategy has been exploited for ligand-positive components. All the information collected from the above strategies can be further processed for computational analysis.

this methodology, sequential lectin affinity columns are used for glycopeptide enrichment, which are further resolved by trimethylsilyl reversed phase HPLC and analyzed by MALDI-TOF or Edman degradation. This method may further be coupled with database searching. Alternatively, another MS based technique termed β elimination followed by Michael addition with dithiothreitol (BEMAD) can also be used for the identification of *O*-GlcNAc-modified sites (23). This *in vitro* methodology relies on mild β elimination followed by Michael addition with DTT. This method is suitable for the quantification of glycosylation by mass spectrometry. Methods to quantify post-translational modification have been recently updated (24).

To conclude, the integration of various MS-based approaches into carbohydrate microarray technologies will generate more ideas for tackling the enormous task of unraveling the huge amount of biological information that is currently being obtained, which will increase the understanding of the molecular machinery of life.

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REFERENCES

1. **Dove, A.:** The bittersweet promise of glycobiology. Nat. Biotechnol., **19**, 913–917 (2001).
2. **Laine, R. A.:** A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05×10^{12} structures for a reducing hexasaccharide: the *isomer barrier* to development of single-method saccharide sequencing or synthesis system. Glycobiology, **4**, 759–767 (1994).
3. **Wang, D., Liu, S., Trummer, B. J., Deng, C., and Wang, A.:** Carbohydrate microarray for the recognition of cross-reactive molecular markers of microbes and host cells. Nat. Biotechnol., **20**, 275–281 (2002).
4. **Wang, D. and Lu, J.:** Glycan arrays lead to the discovery of autoimmunogenic activity of SARS-CoV. Physiol. Genomics, **18**, 245–248 (2004).
5. **Fukui, S., Feizi, T., Galustian, C., Lawson, A. M., and Chai, W.:** Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions. Nat. Biotechnol., **20**, 1011–1017 (2002).
6. **Feizi, T., Fazio, F., Chai, W., and Wong, C. H.:** Carbohydrate microarrays — a new set of technologies at the frontiers of glycomics. Curr. Opin. Struct. Biol., **13**, 637–645 (2003).
7. **Mizuno, M., Noguchi, M., Imai, M., Motoyoshi, T., and Inazu, T.:** Interaction assay of oligosaccharide with lectins using glycosylasparagine. Bioorg. Med. Chem. Lett., **14**, 485–490 (2004).
8. **Fazio, F., Bryan, M. C., Blixt, O., Paulson, J. C., and Wong, C. H.:** Synthesis of sugar arrays in microtiter plate. J. Am. Chem. Soc., **124**, 14397–14402 (2002).
9. **Bryan, M. C., Lee, L. V., and Wong, C. H.:** High-throughput identification of fucosyl transferase inhibitors using carbohydrate microarrays. Bioorg. Med. Chem. Lett., **14**, 3185–3188 (2004).
10. **Banin, E., Neuberger, Y., Altshuler, Y., Halevi, A., Inbar, O., Dotan, N., and Dukler, A.:** A novel linear code® nomenclature for complex carbohydrates. Trends Glycosci. Glycotechnol., **14**, 127–137 (2002).
11. **Park, S. and Shin, I.:** Fabrication of carbohydrate chips for studying protein-carbohydrate interactions. Angew. Chem. Int. Ed., **41**, 3180–3182 (2002).
12. **Houseman, B. T. and Mrksich, M.:** Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. Chem. Biol., **9**, 443–454 (2002).
13. **Ratner, D. M., Adams, E. W., Su, J., O'Keefe, B. R., Mrksich, M., and Seeberger, P. H.:** Probing protein-carbohydrate interactions with microarrays of synthetic oligosaccharides. Chembiochem, **5**, 379–382 (2004).
14. **Adams, E. W., Ueberfeld, J., Ratner, D. M., O'Keefe, B. R., Walt, D. R., and Seeberger, P. H.:** Encoded fiber-optic microsphere arrays for probing protein-carbohydrate interactions. Angew. Chem. Int. Ed., **42**, 5317–5320 (2003).
15. **Guo, Y., Feinberg, H., Conroy, E., Mitchell, D. A., Alwarez, R., Blixt, O., Taylor, M. E., Weis, W. I., and Drickamer, K.:** Structural basis for distinct ligand-binding and Targeting prop-

erties of the receptors DC-SIGN and DC-SIGNR. Nat. Struct. Biol. Mol. Biol., **11**, 591–598 (2004).
16. **Willats, W. G. T., Rasmussen, S. E., Kristensen, T., Mikkelsen, J. D., and Knox, J. P.:** Sugar-coated microarrays: a novel slide surface for the high-throughput analysis of glycans. Proteomics, **2**, 1666–1671 (2002).
17. **Schwarz, M., Spector, L., Gargir, A., Shtevi, A., Gortler, M., Altstock, R. T., Dukler, A. A., and Dotan, N.:** A new kind of carbohydrate array, its use for profiling antiglycan antibodies, and the discovery of a novel human cellulose-binding antibody. Glycobiology, **13**, 749–754 (2003).
18. **Su, S. V., Hong, P., Baik, S., Negrete, O. A., Gurney, K. B., and Lee, B.:** DC-SIGN binds to HIV-1 glycoprotein 120 in a distinct but overlapping fashion compared with ICAM-2 and ICAM-3. J. Biol. Chem., **279**, 19122–19132 (2004).
19. **Nimrichter, L., Gargir, A., Gortler, M., Altstock, R. T., Shtevi, A., Weisshaus, O., Fire, E., Dotan, N., and Schnaar, R. L.:** Intact cell adhesion to glycan microarrays. Glycobiology, **14**, 197–203 (2004).
20. **Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Oka, T., Futai, M., Muller, W. E. G., Yagi, F., and Kasai, K.:** Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. Biochim. Biophys. Acta, **1572**, 232–254 (2002).
21. **Adams, E. W., Ratner, D. M., Bokesch, H. R., McMahon, J. B., O'Keefe, B. R., and Seeberger, P. H.:** Oligosaccharide and glycoprotein microarrays as tools in HIV glycobiology; glycan-dependent gp120/protein interactions. Chem. Biol., **11**, 875–881 (2004).
22. **Hirabayashi, J., Hayama, K., Kaji, H., Isobe, T., and Kasai, K.:** Affinity capturing and gene assignment of soluble glycoproteins produced by the nematode *Caenorhabditis elegans*. J. Biochem. (Tokyo), **132**, 103–114 (2002).
23. **Wells, L., Vosseller, K., Cole, R. N., Cronshaw, J. M., Matunis, M. J., and Hart, G. W.:** Mapping sites of *O*-GlcNAc modification using affinity tags for serine and threonine post-translational modifications. Mol. Cell. Proteomics, **1**, 791–804 (2002).
24. **Raghothama, C. and Pandey, A.:** Absolute systems biology — measuring dynamics of protein modifications. Trends Biotechnol., **21**, 467–470 (2003).
25. **Nilsson, C. L.:** Lectins: proteins that interpret the sugar code. Anal. Chem., **75**, 348A–353A (2003).
26. **Bidlingmaier, S. and Snyder, M.:** Carbohydrate analysis prepares to enter the "omics" era. Chem. Biol., **9**, 400–401 (2002).
27. **Bryan, M. C., Plettenburg, O., Sears, P., Rabuka, D., Wacowich-Sgarbi, S., and Wong, C. H.:** Saccharide display on microtiter plates. Chem. Biol., **9**, 713–720 (2002).
28. **Flitsch, S. L. and Ulijn, R. V.:** Sugars tied to the spot. Nature, **421**, 219–220 (2003).
29. **Hirabayashi, J.:** Oligosaccharide microarrays for glycomics. Trends Biotechnol., **21**, 141–143 (2003).