REVIEW ARTICLE

Glycosylation Pattern of Biotechnologically Produced Proteins - Lectin Array Technology as a Versatile Tool for Screening?

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ABSTRACT

Approximately 50 – 60% of all human proteins are glycosylated. Glycosylation can not only affect the structure of proteins, but also their biological activity, serum half-life, pharmacokinetics, pharmacodynamics, and immunogenicity. For biotechnologically derived proteins, analysis of glycosylation patterns is thus of utmost importance. Standard techniques are based on high performance liquid chromatography, mass spectrometry and capillary electrophoresis. Lectin microarrays are an orthogonal tool, which is very promising for studying glycosylation patterns of intact proteins. However, though the advantages of lectin arrays for the analysis of glycoproteins have been discussed especially in review articles currently only a handful of original publications are available, which are presenting data about therapeutic proteins analyzed with this promising technology. Within this review article, important aspects for analysis of therapeutic glycoproteins are highlighted from the perspective of the lectin array technology. This review article includes generation of cell lines for the production of therapeutic proteins, influence of cell culture conditions on glycosylation, glycosylated antibodies, and their effector functions, glycoengineering, regulatory guidance for biosimilars, and methods for glycosylation analysis with special emphasis on lectin microarrays. The available literature proves that especially the lectin array technology is an upcoming tool for screening the glycosylation pattern of biotechnologically derived proteins. The technology is also versatile and more applications will be utilized in the near future for example for biomarker resarch and application as a diagnostic tool.

Keywords: Glycosylation, lectin microarray, glyco-profiling, glycan analysis, biosimilars, recombinant glycoprotein, monoclonal antibody, regulatory guidance, hemagglutinin; lectin histochemistry

1. Introduction

The major characteristics of a protein are determined first by its primary structure and amino acid sequence. The next levels are secondary, tertiary, and quaternary structures. Furthermore, protein features can also be modified by posttranslational modifications including glycosylations, an enzymatic process that attaches glycans to proteins, lipids, or other organic molecules. Approximately 50 – 60% of human proteins get some kind of glycosylation usually by the addition of N- or O-linked glycans.[1, 2] Differences in glycosylation patterns exist at every level of biological organization, between species, tissues, cell types, and proteins within the same organism.[3] In eukaryotic cells, glycans are produced and maturated in the endoplasmic reticulum and Golgi apparatus and are normally a mixture of different N-linked and O-linked structures.[4] Glycosylation not only affects the structure of proteins, but also their biological activity, serum half-life, pharmacokinetics (PK), pharmacodynamics (PD) and immunogenicity.[5, 6]

Therapeutic proteins are the most promising class of glycosylated biopharmaceuticals due e.g. to successful treatment of cancer and immune disorders. Biopharmaceuticals currently represent the fastest growing sector of the pharmaceutical industry and there is a tremendous rush by many companies worldwide to develop biosimilar versions of innovator products.[7, 8]
Analysis of antibody glycosylation patterns is thus of utmost importance. Standard physico-chemical techniques are based on high performance liquid chromatography (HPLC), mass spectrometry (MS), and capillary electrophoresis (CE).[8, 10] The application of lectins as a class of molecules that can specifically bind carbohydrate-protein structures has evolved in the last years in combination with microarrays as a promising additional tool for studying the glycosylation patterns of proteins.[11] However, though the advantages of lectin arrays for the analysis of glycoproteins had been discussed in several peer-reviewed articles to date only a handful of original publications are available, which are presenting data about therapeutic proteins analyzed with this promising platform technology. In this review, we thus want to highlight aspects for the analysis of therapeutic glycoproteins from the perspective of the lectin array technology.

2. Eucaryotic Cell Lines for the Production of Therapeutic Proteins

Eucaryotic cell lines have emerged as a preferred source for the production of human therapeutic proteins. Significant differences in the glycosylation pattern of recombinant proteins do not only exist when expressed in yeast, insect and mammalian cells but also between different mammalian cell lines.[1, 2] Even individual transgenic animals showed slight inter-individual differences.[12]
Human cell lines seem to be the most genuine and logical choice for biotechnological production[13] but are nowadays rarely employed. As glycosylation profiles of eukaryotic expression systems differ from human physiological pathways a variety of glycosylation strategies have been proposed for humanizing the glycosylation pathways.[14] In this respect, also differences in modifications of recombinant mAbs in comparison to those of endogenous immunoglobulin G (IgG) molecules were frequently observed.[15] In order to adequately select a cell line for the production of a therapeutic protein a number of aspects need to be considered including cell culture conditions.

3. Cell Culture Conditions and Influence on Glycosylation

Glycosylation and optimization of cell culture processes have many implications for the

biotechnology industry.[16, 17] The degree of glycosylation depends in first line on the cell line itself due to differences in activities of cellular metabolism and / or expression of glycosyltransferase enzymes. In addition, every single cell-culture condition may influence the glycosylation pattern including the mode of culture operation, incubation conditions, changes in supplements, growth rate, and amount of generated protein.[3, 18] If properly controlled, the quality of a recombinant product in terms of O- and N-linked oligosaccharides can be stable.[19] The majority of reports, however, indicate that even minor differences in growth conditions can result into major differences of glycosylation patterns.

Aghamohseni et al. evaluated the impact of operating conditions on the glycosylation pattern of humanized camelid (= single domain) mAb and there was a tradeoff between cell growth, the resulting productivity and the achievement of desirable glycosylation levels.[20]

Ivarsson et al. investigated the effect of single and combined chemical and mechanical stress parameters on the glycan micro-heterogeneity of an IgG1 antibody[21]. Within a pH range of 6.8 to 7.8 differences in galactosylation and sialylation of nearly 50 % were observed. Variation of dissolved oxygen tension between 10 to 90% air saturation resulted into a maximum variability of 20 % in galactosylation and 30 % in sialylation.

Amino acids as basic supplements of mammalian cell culture feeds have also effects on the glycosylation pattern.[22] The nutrient levels and the concentrations of byproducts such as ammonia and the adaption to glutamine-free growth have been identified as very significant influence factors as well.[23, 24]

Among further examples for the influence on glycosylation patterns are osmolality levels and extending culture duration[25], the modulation of antibody galactosylation through feeding of uridine, manganese chloride, and galactose[26], or addition of glucocorticoids in a dose- and time-dependent manner.[27]

4. Glycosylated Antibodies and Effector Functions

Different glycosylation patterns must not invariably result into changes of features.[28] However, in general, carbohydrates attached to therapeutic glycoproteins directly affect product quality, safety, and efficacy and it is well known that serious adverse events can be caused by some carbohydrates.[29]

A typical example for severe influences of glycosylation pattern on effector functions on proteins are mAbs.[30, 31] The majority of oligosaccharides of human and recombinant IgGs include core-fucose. In most cases, the levels of terminal galactose and bisecting residue are higher in human IgG compared with recombinant IgG molecules and a-glycosylated antibodies and high mannose are usually present at much higher levels in recombinant mAbs compared with human IgG.[30]

Importantly, carbohydrates like terminal galactose residues, bisecting GlcNAc and core fucose have a critical impact on mAb mediated effector functions like antibody-dependent cellular cytotoxicity (ADCC).[32] Core fucose reduces IgG antibody binding to IgG Fcγ receptor IIIa resulting in decreased ADCC activities,[30] while the presence of a terminal galactose or bisecting residue only has a subtle effect on receptor binding and ADCC.[33, 30]

Mannosylated glycans and sialic acid N-acetylneuraminic acid (NANA) can impact PK, and lower levels of galactose reduce complement-dependent cytotoxicity (CDC) activity.[30] Furthermore, modifications that are not common to endogenous IgG molecules pose a higher risk of immunogenicity.[15, 30]

Regarding the clinical efficacy of therapeutic mAbs, those fully lacking core fucosylation have attracted attention as next-generation approaches (second line products) because of their improved ADCC activity.[34, 35] The first glyco-engineered antibody with enhanced ADCC to reach the market (in Japan), mogamulizumab / Poteligeo®, was regarded as a landmark.[36]

Glycosylation constitutes a critical quality attribute for therapeutic proteins and for optimal efficacy and safety a framework for designing the quality target product profile is required.[4] Moreover, glycan patterns of individual mAbs must be adequately analyzed at every process step throughout the product life cycle including batch-to-batch consistency. Drastic effects on biological functions and *in-vivo* recovery are not only restricted to mAbs, but can also be observed on many other therapeutic glycoproteins, just naming recombinant coagulation factor IX.[37] If biosimilars are developed, structural and activity related comparability to the innovator must be demonstrated as well.[8, 38]

5. Glycoengineering and Quality by Design

Quality by design (QbD) is a process to ensure product quality by integrating it into the manufacturing process of biopharmaceutical products.[39, 40] Accordingly, glyco-engineering of expression platforms is an important strategy to improve biopharmaceuticals.[41] A classical approach for QbD is to analyze cell culture medium components and supplements affecting the quality attributes.[42] Modulation of sialylation patterns through overexpression of sialyltransferases might be just an example to produce desired glycoforms.[43] To avoid time-consuming experimentation for clone identification and optimization of biosimilars, various computational methods to predict an optimal glycosylation profile can be applied.[44, 45,46]

6. Regulatory Guidance for Biopharmaceuticals and Biosimilars

In respect of glycosylation profiles, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) issued two guidelines detailing the specifications of biopharmaceuticals[47] as well as comparability of such structure profiles during process scale-up and changes in manufacturing process.[49] According to these guidelines, glycosylation is a key critical quality attribute and subsequently should be controlled and monitored throughout the development and production processes of therapeutic proteins.

According to the European Medicines Agency (EMA), a biosimilar is a copy version of an already approved biopharmaceutical drug with (very) similar biologic activity, physicochemical characteristics, efficacy, and safety. To ensure similar efficacy and safety comparability should be analyzed at quality, preclinical and clinical level.[50] Basic regulatory guidance is laid down by the EMA in several issues[51, 52, 53, 54] For assessment of biosimilarity, FDA recommends a stepwise approach for demonstrating biosimilarity between a proposed biosimilar product and a biological originator (innovator) product.[55, 7, 56] The defined regulatory requirements for biosimilars in various countries across the world were reviewed by Chugh et al.[57]

7. The Rituximab Story

An example makes it clear that a biosimilar should never be developed without knowing the status of the glycosylation pattern in comparison to the originator molecule. Rituximab (Rituxan™, MabThera™) is a chimeric IgG mAb directed against CD20 surface protein. One biosimilar in development showed higher receptor affinity and higher ADCC activity, therefore EMA has advised the applicant to adjust the manufacturing process. After thorough analysis, the primary amino acid sequence of the biosimilar was shown to be identical, and secondary and tertiary structures of the proteins were indistinguishable. However, proportions of some glycosylations were slightly different. The development was restarted and a modified manufacturing process finally directed the oligosaccharide composition within the variability of the originator.

For development of the rituximab biosimilar GP2013 post-translational modifications and bioactivities of GP2013 versus the originator rituximab were engineered and monitored to ensure similar pharmacological profiles.[58] In another study comparing rituximab and biosimilars, N-glycosylation profiles obtained from three batches of the biosimilar and the reference product showed quantitative variations, although N-glycans were qualitatively similar.[59]

8. The Infliximab Story

Remsima™ (infliximab), a tumor necrose factor α blocker, is the first biosimilar mAb approved by EMA and FDA. The originator product is Remicade®. Remsima™ has higher levels of soluble aggregates, C-terminal lysine truncation, and fucosylated glycans. Glycosylation patterns were extensively studied. With forced degradation studies it was shown that infliximab's primary sequence largely defines the protein instabilities and glycosylation differences had limited influence.[60] In another infliximab study the biosimilar Remsima™ and the originator Remicade® were compared and in general, the amount of glycans was consistent in both, with no new glycans detected.[61] Remicade®, and biosimilar products Flixabi®, Renflexis® and Remsima®, and Inflectra® were also compared and correlated with effector functions.[62]

9. The Cetuximab Story

Cetuximab is produced in SP2.0 murine myeloma cells and is N-glycosylated in the Fc and Fab domains of the antibody. 21 distinct oligosaccharide structures were observed[63] and a comprehensive profile of the glycoforms of the EMA-approved cetuximab is available.[64] The analysis of the glycosylation pattern of cetuximab makes especially sense, because a high prevalence of hypersensitivity reactions associated with glycan structures were reported and some of the glycoforms were demonstrated to be responsible for these reactions as well as anaphylaxis.[65] The glycan profiling of a potential biosimilar candidate of cetuximab revealed that the major glycan moieties in the biosimilar were in agreement with the innovator.[66]

10. Glycosylation Analysis of other Therapeutic Proteins

Not in every case a distinct gycosylation pattern results into significant differences. A biosimilar of trastuzumab and its reference product exhibited a high degree of similarity for a number of evaluated features including glycosylation profiles.[67] HS628, a biosimilar of originator tocilizumab (Actemra®) had a similar glycosylation patterns as the originator tocilizumab and no modified effector functions were observed.[68] For adalimumab / Humira® product quality data from more than a decade of manufacturing across multiple production sites and through a series of manufacturing scale changes were compiled.[69] In this case, the glycosylation patterns have remained remarkably consistent. N-glycosylation consistency was observed in several production batches of nimotuzumab (a humanized anti-EGF-R antibody) that lasted between 68 and 150 days.[70] Also biosimilars of trastuzumab were analyzed in detail.[71, 72] Comprehensive glycosylation profiling confirmed that proportion of individual glycans was different between biosimilar and the innovator, although the number and identity of glycans were the same.[71]

However, issues with glycosylation patterns of other therapeutic antibodies, which have occurred in the past have already drawn attention towards a thorough analysis of glycan structures and potential clinical implications.

Recombinant human follicle-stimulating hormone (r-hFSH) is widely used in fertility treatment of women. The biosimilars Bemfola® and Ovaleap® showed differences in pregnancy rates and ovarial hyperstimulation syndroms in comparison to FSH originator product

follitropin alpha / Gonal-f™. Accordingly, it was not recommended by some physicians to interchange or substitute innovator and biosimilars in clinical practice.[73] This could have been avoided, because previously Gonal-f™ has been already compared to a potential biosimilar candidate and it was demonstrated that two r-hFSH preparations have a different glycosylation pattern. N-terminal glycosylation site of the β-chain of the biosimilar contained a higher percentage of tri- and tetra-antennary glycans and of N-acetyllactosamine repeats as compared to Gonal-f™.[74]

The site-specific glycosylation profile and batch-to-batch variability of *in-vivo* bioactivity of Bemfola® with its reference product GONAL-f™ was also analyzed by Mastrangeli et al.[75] A lower proportion of bi-antennary structures, and a higher proportion of tri-antennary and tetra-antennary structures was observed at Asn52. This, together with the higher bioactivity and higher batch-to-batch variability of Bemfola®, could partly explain differences in clinical outcomes.

Glycosylation of recombinant human erythropoietins (rhEPOs) is significantly associated with drug's quality, structure and potency. Glycoform profilings of biosimilar and innovator EPO products showed characteristic glycoform profiles with respect to sialylation, glycan size, O-acetylation of sialic acids and O-glycosylation.[76, 77] An in-depth characterization of glycosylation of a candidate biosimilar of CTLA4-Ig, a highly glycosylated therapeutic fusion protein containing multiple N- and O-glycosylation sites, was also strongly recommended.[78]

A comprehensive glycosylation study was conducted with several antibodies in parallel, i.e. batch-to-batch consistency of the N-glycosylation of infliximab, trastuzumab and bevacizumab was analyzed.[79] All batches of the therapeutic glycoproteins varied considerably, especially in galactosylation. The authors therefore suggested to establish threshold values for batch-to-batch N-glycosylation variations in order to regularly test batch-to-batch glycosylation consistency. In these cases, however, significantly different N-glycosylation profiles did not result into significant variations in biological activity.

In summary, though not always differences in glycosylation structures invariably end into different measurable biological or therapeutic features, recent studies tend to the recommendation not to develop a biosimilar without a thorough comparison to glycosylation patterns of the originator molecule. In view of such issues and reffering to the increasing demands on knowledge of glycan structures, it is not surprising that during the last few years the analysis of glycovariants of biosimilars in comparison to their originators got considerable interest and in addition to well-established methods, a number of improved or new technologies were developed for the analysis of glycosylation structures of proteins.

11. Methods for Glycan Analysis

No universal method for a rapid and reliable identification of glycan structures is currently available and therefore the specific glycoprotein to be analyzed must dictate the best method or combination of methods, especially whether N- and / or O-glycan analysis will be performed.[80, 4, 8] Basic analytical techniques used for glycoprotein analysis include HPLC, CE, MS, and high-throughput analytical methods based on microfluidics.[80] Chemical and enzymatic releasing methods of glycans from glycoproteins and chemical reactions for the derivatization of glycans, and chemical labeling methods are also needed as supporting tools.[82] IEF, IEX, or CE alone or in combination is commonly applied for heterogeneity in sialic acids on intact glycoproteins, HPLC for quantitation of amounts of released oligosaccharides, and MS coupled with HPLC for characterization of glycosylation site(s) occupancy and carbohydrate structures.[8]

Further developments of well-established methods are presented from time to time. For

example two ultrafast methods for antibody glycan analysis that involve the rapid generation and purification of glycopeptides in either organic solvent or aqueous buffer followed by label-free quantification using matrix-assisted laser desorption / ionization-time of flight mass spectrometry.[83] Both methods yield to N-glycan profiles of test antibodies similar to those obtained by traditional methods in shorter assay time and in a high throughput format in 96-well PCR plates. Obviously, there is a need for further simple, high-speed, and low cost methods that may enhance research, process development, batch-to-batch analysis, and comparison for novel mAbs and biosimilar products.

12. Lectin Microarray Development

In addition to "classical" HPLC and MS methods[84, 85, 86], a new promising technology for the analysis of glycosylation pattern is the lectin microarray (Figure 1).

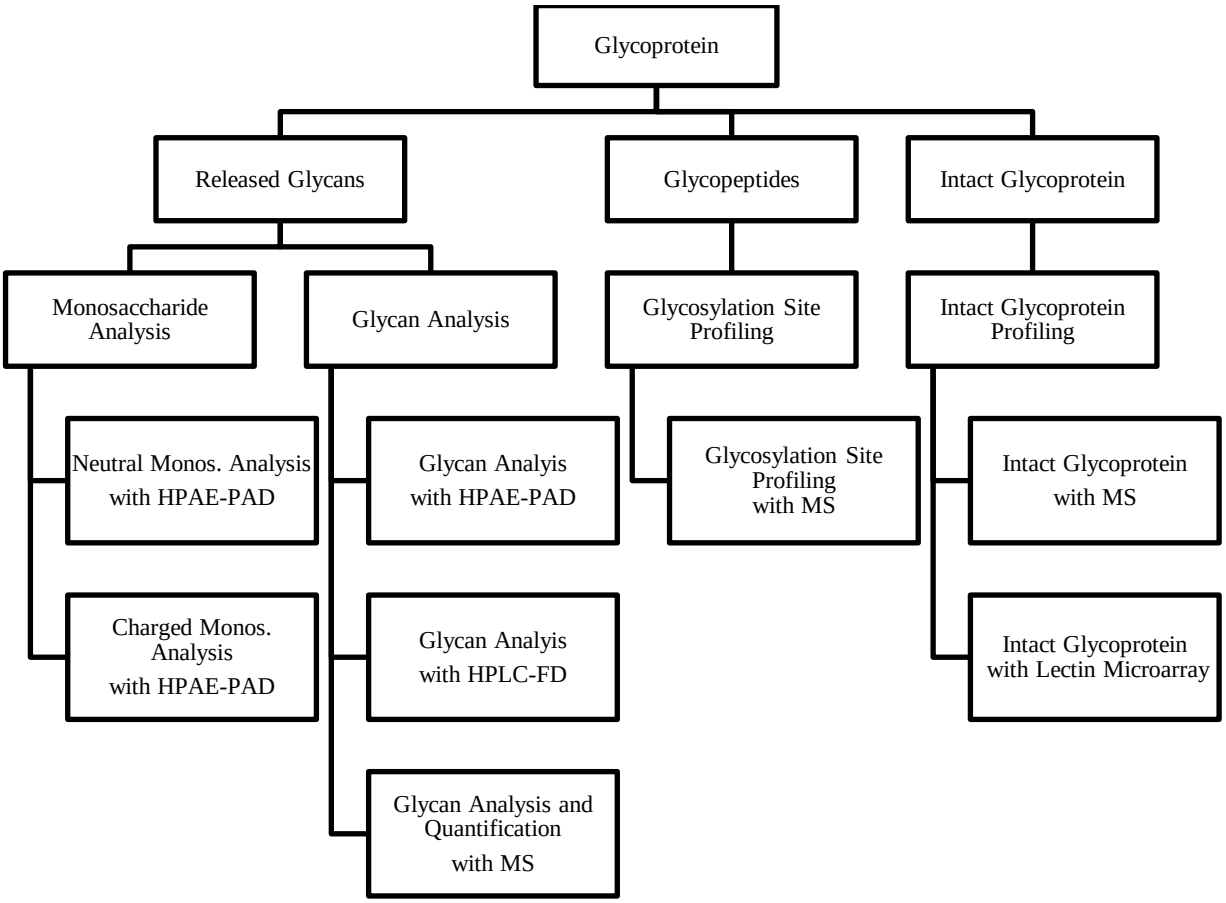

Figure 1: The figure gives a simplified overview for the measurement of glycoproteins. Glycosylated proteins can be either treated or digested to release the glycans or their peptides respectively, or can be analyzed on the intact glycoprotein. Dependent on the necessitiy of quanitfication different analytical methods can be applied. For the comparison of glycopatterns the lectin microarray provides an orthogonal way to analyse the intact protein.

Lectin microarrays were first reported in 2005[87, 88] and are prepared by immobilizing various lectins on a solid surface. These sugar-binding proteins are generally classified into five groups, according to the monosaccharide for which they exhibit the highest affinity: mannose, galactose / N-acetylgalactosamine, N-acetylglucosamine, fucose, and sialic acid.[87] The microarray procedure is based on an evanescent-field fluorescence-detection principle, which allows sensitive, real-time observation of multiple lectin-carbohydrate interactions under equilibrium conditions.[87, 89] The method allows quantitative detection of even weak lectin-carbohydrate interactions with a dissociation constant of $K_d > 10^{-6}$ M. Analytes including glycoproteins, whole cells, or bacteria are labelled with a fluorescent dye or antibody before loading onto a commercially available lectin microarray containing up to 45 lectins (**Figure 2**).

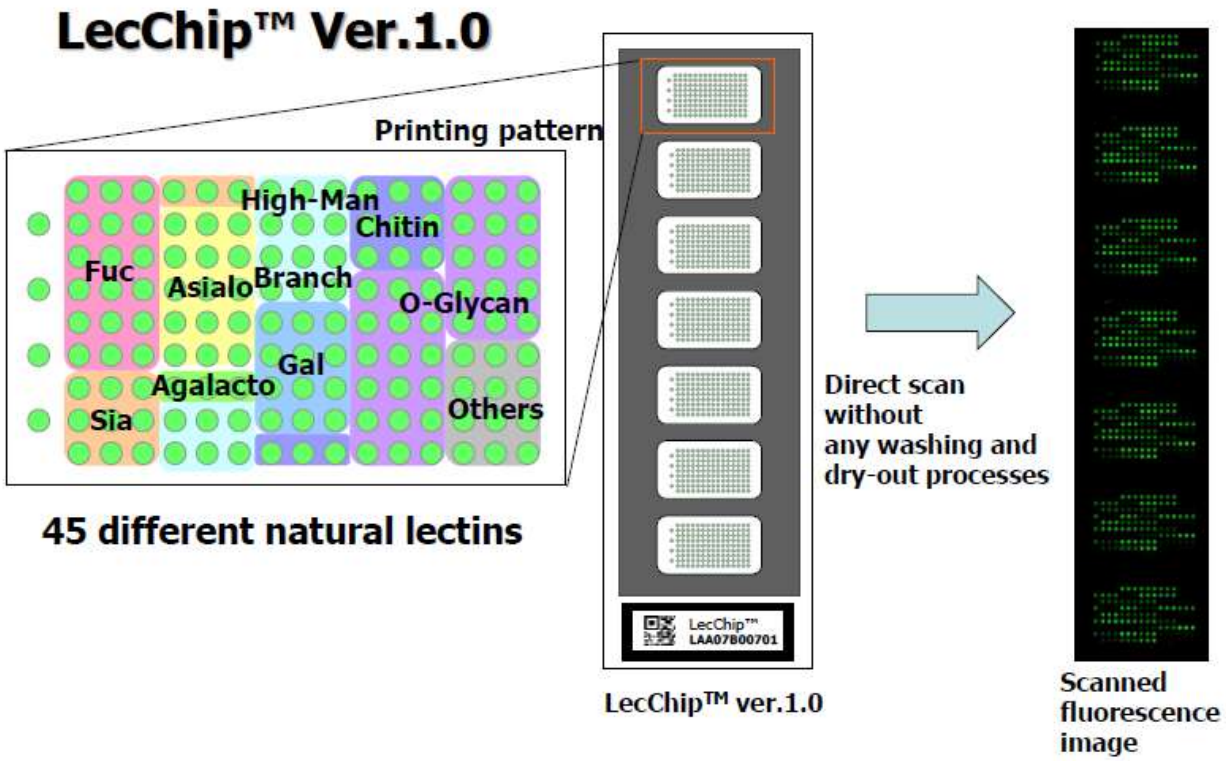

Figure 2: Layout of a lectin chip (LecChip™ by Glycotechnica Ltd; the figure is a courtesy of Masao Yamada PhD, Glycotechnica Ltd.).
(A) The chip has 4 position marker on the left and 45 different lectins clustered in groups for specific carbohydrate binding. Each of the lectins is printed in triplicate. (B) Each chip has 7 wells, which allows either a concentration dependent measurement or technical replicates. (C) After incubation with fluorescent dye Cy3 the chip is analyzed in the scanner (GlycoStation Reader). For analysis intensity of the dots, the scanned picture is transformed into numbers which allows to generate the glycosylation pattern of the analyte.

Depending on the carbohydrate structures attached to the analyte, binding of different protein structures to certain, specific lectin species will occur. There is no washing step required, and the chip is analyzed by a confocal type fluorescence scanner. Other lectin microarrays formats for the high-throughput analysis of glycosylation are reported as well.[90]

Fabrication and detection strategies of lectin arrays and their applications were reviewed by Hu et al.[91], Huang et al.[92], and Hirabayashi et al.[93] Several options of microarray platforms including glycoprotein arrays, glycan arrays, lectin arrays, and antibody combined lectin arrays are described.[94] For improvement of sensitivity, lectins were chemically coupled to fluorescent dye coated microbeads and the detection was carried out three dimensionally.[95] With this method, a limit of detection of 1 pM was reached for lectin Ricinus communis agglutinin 120. A lectin-based enzyme-linked immunosorbent assay (ELISA) to quantify terminal glycan moieties was also described.[96] A new integrated and automated microfluidic lectin barcode platform may improve and speed up the performance of lectin arrays.[97]

Lectin microarrays especially hold a promise of enabling glycomic profiling of cancers in a fast and efficient manner and already gained considerable interest in various cancer types.[98] However, this seems to be not the end of possibilities for supporting diagnostic decisions. Recently an analysis of glycosylation patterns in Alzheimer's disease-affected brain regions as well as in Alzheimer's disease patient serum was presented.[99] Differences of glycan levels in protein O-GlcNAcylation and N- / O-glycosylation between patients and healthy individuals and brain region-specific glycosylation-related pathology in patients were observed.

Glycoproteins are potentially important biomarkers of many diseases and also therapeutic targets. Additional applications for lectin arrays can be explored for example on the glycosylation profile of tear fluid.[100, 101] Another interesting field can be the glycoprofile of human milk oligosaccharides as an orthogonal method to CaR-ESI-MS.[102, 103] Also during spermiogenesis post-translational modifications and glycosylation play an important role in the reproduction system.[104, 105, 106]

13. Lectin Array vs other Glycan Profiling Methods

Results of commercially available lectin arrays are semi-quantitative and for accurate and specific carbohydrate identification standard methods like HPLC, MS and CE should still be considered in addition.[107] The potential utility of lectin-based microarrays for high throughput glycan profiling was compared with pros and cons of major types of established analytics for use in determining glycan features.[8] One of the major advantages of lectin microarrays appeared to be direct measurements in an intact protein without the need of clipping glycans from the protein backbone. Thus, this methodology was suggested to be applied as a complementary tool for characterization of protein glycosylation. The major advantages of microarrays are analytical sensitivity and relatively high sample through-put, and only a very small amount of sample is needed for analysis.[93]

14. Lectin Arrays for Glycosylation Analysis of Therapeutic Proteins

In general, lectin array technology has been already applied to study implication of glycosylation in cancer, bacteria, fungi, stem cells, sperm, and diabetes.[93] However, though the advantages of lectin array analysis are obvious, it was up-to-date hardly used for glycosylation analysis of therapeutic proteins.[8] Only a few studies with successful applications of lectin arrays were published. The glycosylation pattern of a recombinant CTLY4-IgG fusion glycoprotein expressed in CHO cells was determined with a lectin array and compared to traditional negative mode capillary LC-MS of released oligosaccharides.[108] The glycosylation pattern including information about sialylation, the presence of reducing terminal gal β1-, terminal N-acetylglucosamine β1-, and antennary distribution was comparably with both methods applied.

A lectin array-type method specifically designed for the study of recombinant therapeutic interleukin-7 was employed for a lot-to-lot comparison of different batches of the protein produced in CHO cells.[109] The method allowed analysis of glycans motifs, distribution of glycoforms, and detection of potential immunogenic glycans.

The authors concluded that lectin array technology is of considerable interest for the development of therapeutic recombinant glycoproteins and particularly relevant for a first informative study of unwanted glycans during process development.

Porcine and human fibrinogen glycoproteins were analyzed with a specifically developed nine-lectin screen.[110] The observed spectra of lectin-protein specific binding rates allowed to distinguish between glycosylation of the porcine and human fibrinogens.

The N-linked glycosylation of four lots of a human therapeutic mAb was assessed by three orthogonal chromatographic methods and compared to a lectin microarray.[111] Despite the orthogonality of the methods, a high degree of consistency in the types and amounts of N-linked glycans and between all four analysis methods was observed. Moreover, the glycosylation analyses provided also complementary and corroboratory qualitative and quantitative information.

Until now the most comprehensive study around the utility of lectin arrays for the assessment of therapeutic glycoproteins was conducted by a research group within the US-FDA.[38] Using a commercially available lectin chip containing 45 lectins the binding patterns of a broad variety of 15 therapeutic proteins, including 8 mAbs was assessed. The antibodies were bevacizumab / Avastin®, trastuzumab / Herceptin®, adalimumab / Humira®, infliximab / Remicade®, rituximab / Rituxan®, omalizumab / Xolair®, cetuximab / Erbitux® and the fusion protein etanercept / Enbrel®, the other proteins were from the groups of recombinant therapeutic cytokines and enzymes, and of human transferrin proteins. In summary, lectin binding signals were generally consistent with the previously known glycan patterns for the respective glycoproteins. The lectin microarray was especially sensitive to variations in terminal carbohydrate structures such as galactose versus sialic acid epitopes. This study clearly showed that lectin microarrays are useful tools for screening glycan patterns of therapeutic glycoproteins.

In addition to screen glycan structures of therapeutic proteins, lectin arrays can be, for example, a perfect tool to predict certain effector functions and activity or potency of therapeutic proteins. In a recent study, lectin microarray technology was applied to compare the glycosylation pattern of a mAb expressed in SP2.0 cells to an ADCC-optimized de-fucosylated variant expressed by a plant expression system (MB314).[112] A fucose indicative lectin-binding pattern correlated with increased MB314 binding to CD16 whose affinity is mediated through core fucosylation and stronger ADCC. The expected positive correlation of increased ADCC to the de-fucosylated variant demonstrated that lectin binding data can be used as a surrogate parameter to predict biological functions.

15. Conclusion

According to recent literature, the lectin microarray is a rapid tool for profiling carbohydrate structures of therapeutic glycoproteins especially for mAbs. The analytical sensitivity and sample throughput of lectin microarrays is relatively high and only a small amount of sample is needed for analysis. The curently available data – mostly for therapeutic glycoproteins (antibodies) clearly show that lectin binding signals are generally consistent with the previously known glycan patterns. The lectin array technique has advantages in monitoring the glycosylation pattern during process development for recombinant proteins, which depend on various parameters such as medium feeds, metal ions, and harvest time. Results are semi-quantitative,

and, for accurate and specific carbohydrate identification, standard methods such as HPLC, MS, and CE will be still applied in parallel in order to get full scope of information.

The question of this review whether lectin array technology maybe a useful tool for screening the glycosylation pattern of biotechnologically produced proteins can be answered with a strong ”yes”. This technology is also versatile and more, new applications will be utilized in the near future.

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Author contributions

Klaus Zimmermann wrote the manuscript together with Markus Roucka with valuable input from Markus Fido.

Conflicts of Interest

The authors declare no conflicts of interest.

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