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Lectin-Array Blotting: Profiling Protein Glycosylation in Complex Mixtures

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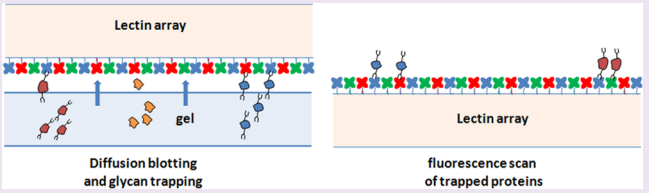
Supporting Information

ABSTRACT: By combining electrophoretic protein separation with lectin-array-based glycan profiling into a single experiment, we have developed a high-throughput method for the rapid analysis of protein glycosylation in biofluids. Fluorescently tagged proteins are separated by SDS-PAGE and transferred by diffusion to a microscope slide covered with multiple copies of 20 different lectins, where they are trapped by specific carbohydrate protein interactions while retaining their relative locations on the gel. A fluorescence scan of the slide then provides an affinity profile with each of the 20 lectins containing a wealth of structural information regarding the present glycans. The affinity of the employed lectins toward *N*-glycans was verified on a glycan array of 76 structures. While current lectin-based methods for glycan analysis provide only a picture of the bulk glycosylation in complex protein mixtures or are focused on a few specific known biomarkers, our array-based glycoproteomics method can be used as a biomarker discovery tool for the qualitative exploration of protein glycosylation in an unbiased fashion.



Glycosylation is the single most important posttranslational modification and is known to influence protein structure, function, and biological activity.[1,2] Glycans have been shown to play an important role in many biological processes such as cell differentiation, development, fertilization, cell adhesion, and more general cell−host and cell−pathogen recognition.[3] Therefore, glycosylation is a reflection of the physiological state of a cell and is altered in many types of disease including cancer, infection, and autoimmune or neurodegenerative disorders, suggesting a stronger role for prognostic glycan biomarkers in the near future.[3−5] Proteins can be glycosylated *via* asparagine (*N*-glycans) or threonine/serine linkages (*O*-glycans). They often have more than one glycosylation site which, occupied by different glycan structures, can give rise to up to hundreds of possible glycoforms for a single glycoprotein. In addition, individual glycans can be further modified by acyl, sulfate, phosphate, or other groups.

It is no surprise then that technologies for profiling the complex glycoproteome are still in their infancy. A comprehensive structural glycan analysis of a single glycoprotein by mass spectrometric methods is currently possible albeit very time-consuming. However, only limited information regarding, *e.g.*, glycosylation sites or single monosaccharides such as *O*-GlcNAc, can be obtained on a glycoproteome level.[6] This is mainly due to the extensive and time-consuming sample preparation required before glycans can be analyzed in the mass spectrometer. After glycoprotein purification or enrichment by lectin affinity chromatography, electrophoretic separation or immunoprecipation,[1,7] and protein digestion, the glycans are enzymatically or chemically released and often derivatized for quantitative analysis. The large majority of glycan analyses performed are restricted to mammalian *N*-glycans, which are easily cleaved off by the peptide-glycosidase F (PNGase F), while a focus on *O*-glycans, non-mammalian *N*-glycans, or other glycan classes has to deal with less established or less general means of cleavage prior to their analysis.[8]

Lectin-based profiling approaches, on the other hand, are well suited for the rapid screening of changes in protein glycosylation, and both *O*- and *N*-glycosylation on the intact glycoprotein are accessed without need for prior glycan cleavage or further derivatization.[9−11] Lectins are carbohydrate binding proteins often derived from plant seeds, which bind weakly but highly selective glycan epitopes found on glycoproteins.[12] They have become indispensible tools in glycobiology for glycan profiling, imaging, and enrichment since their discovery over five decades ago.[9,13]

Single and multilectin affinity chromatography[14,15] has been employed extensively for the separation and profiling of complex glycoprotein mixtures, and more recently lectin arrays have provided a miniaturized high-throughput solution to the profiling of changes in protein glycosylation in cells or complex protein mixtures.[16−19] By using lectin arrays, differences in glycosylation have been studied in bacteria,[20] stem cells,[21−23] and breast cancer cells.[24] However, without prior protein fractionation,[17,19,25] *e.g.*, by electrophoretic or chromatographic methods, lectin arrays provide only information regarding the overall glycosylation pattern of a complex protein mixture.

Received: May 28, 2012
Accepted: August 7, 2012
Published: August 7, 2012

   dx.doi.org/10.1021/cb300262x | *ACS Chem. Biol.* 2012, 7, 1729−1737

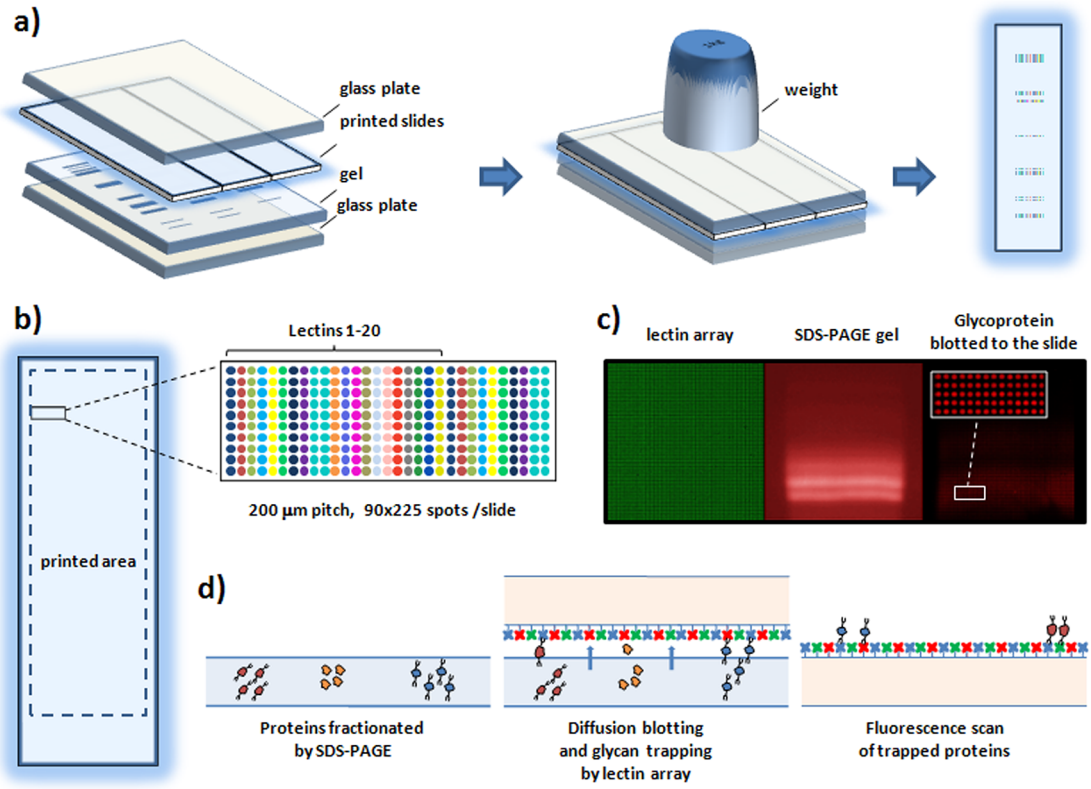


Figure 1. Working principle of lectin array blotting. (a) Workflow lectin array blot. (b) Array design. (c) Left: printed ConA array. Center: fluorescent gel image. Right: fluorescent image of transferred and trapped RNase B bands. (d) Schematic presentation of localized glycoprotein trapping on glass slide *via* specific carbohydrate lectin interactions.

To overcome this limitation, innovative recent approaches that use arrays for the lectin-based analysis of individual glycoproteins in a complex matrix, such as serum or urine, have been developed. These include antibody overlay arrays,[26,27] glycoprotein arrays,[28] or antibody sandwich arrays.[29] These methods, however, either require a laborious multidimensional protein fractionation and subsequent arraying[30] or are limited to the analysis of only a few selected proteins. Often only a fraction of the glycoproteome will show disease-related changes in glycosylation that can be covered up by more abundant proteins in traditional lectin arrays or might not show up at all in antibody overlay or antibody sandwich approaches. Especially for the discovery of new potential disease markers a rapid unbiased screening method that generates a simple fingerprint of protein glycosylation and is highly sensitive to changes is still lacking.[6,11]

Lectin blots[31−33] and 2-D tandem lectin-affinity electrophoresis[34] are simple but very effective methods for profiling entire glycoproteomes, combining lectins as glycan detection probes and gel electrophoresis for protein fractionation, but the information obtained is usually limited to the interaction of the proteins with one or two lectins.

To obtain a more detailed picture of the glycan epitopes present in a protein, a glycan binding profile with a panel of lectins with a broad range of specificities in a single experiment would be necessary. We now have developed a high-throughput method for the rapid analysis of protein glycosylation in biofluids that combines electrophoretic protein separation with lectin-array-based glycan profiling in a single experiment.

RESULTS AND DISCUSSION

The high-throughput tool for profiling protein glycosylation in protein mixtures here described combines lectin arrays with polyacrylamide gel phase electrophoresis to a novel blotting technique. Fluorescently tagged proteins are separated by conventional SDS-PAGE and then transferred onto a glass slide covered entirely with multiple copies of micrometer size lectin arrays by bringing gel and glass slide into contact, a process that has been described as diffusion blotting[35] (Figure 1a).

Only glycoproteins are trapped on the slide by specific lectin−carbohydrate interactions, retaining their relative locations on the gel (Figure 1d). After washing off unbound material, the slide is scanned and lectin affinity profiles are obtained for every gel band. The fluorescence intensity of individual spots is a direct measure for the affinity between a present carbohydrate epitope and the spotted lectin. In other words, our method provides up to 20 lectin blots in a single experiment, a treasure trove of structural information for any complex protein mixture, in a fraction of time and with a minimum amount of reagents.

The interpretation of the lectin binding profiles in terms of protein glycosylation is obviously dependent on a concise knowledge of the individual lectin binding specificities in the first place. Currently, more than 100 lectins, covering a broad range of glycan epitopes, are commercially available for the fabrication of lectin arrays. Although the monosaccharide binding specificity of these lectins, usually in the high millimolar range, is known, detailed knowledge of their glycan binding pattern, particularly toward more complex structures, is scarce. Binding to oligosaccharides, which often exhibit multiple copies of the bound epitope, can show an increase in affinity of up to 3 orders of magnitude or a decrease if access to the epitope is hidden in a

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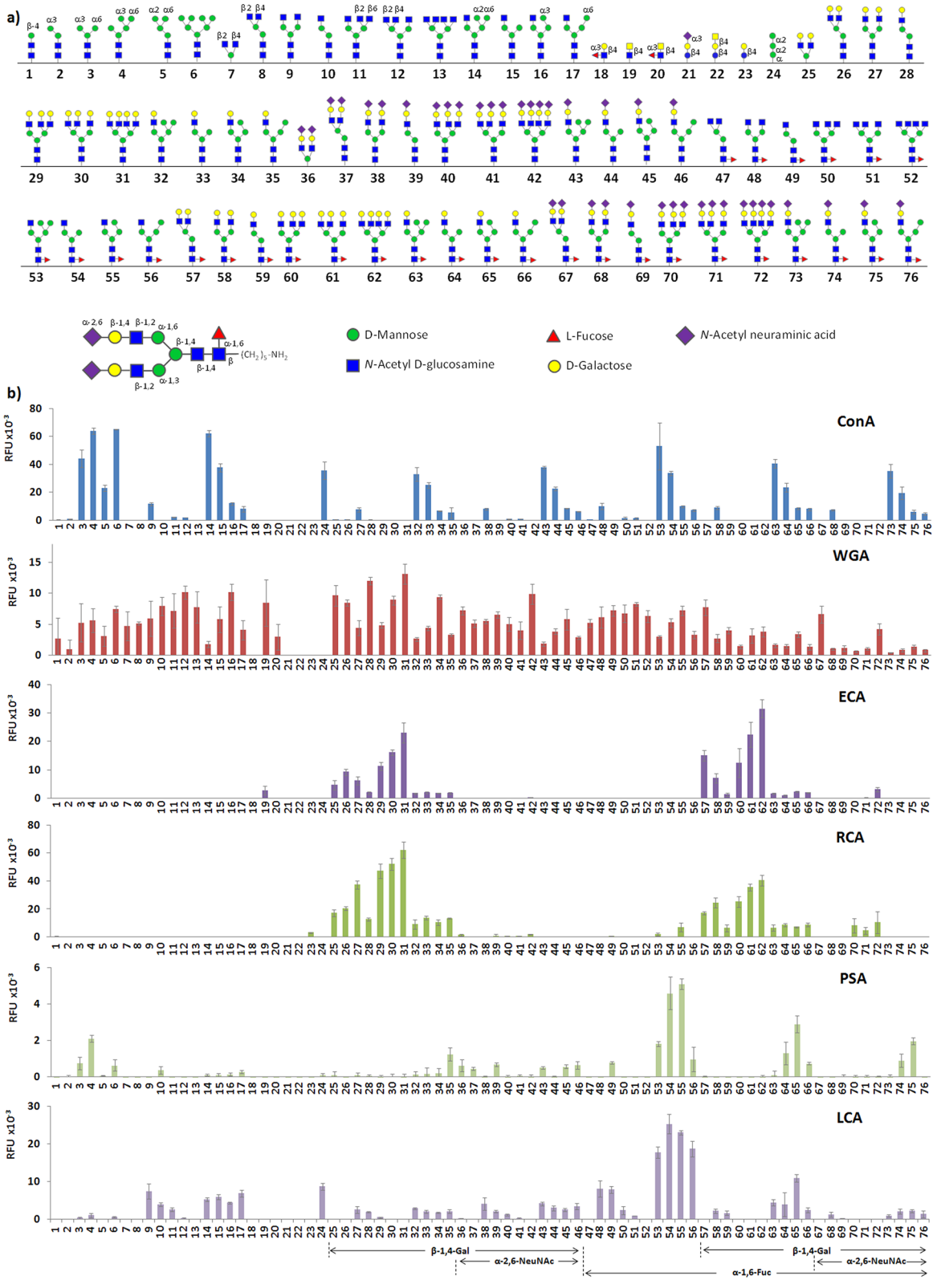

Figure 2. Binding of lectins on synthetic glycan array. (a) Pictogram representation of glycans included in the array. (b) Histograms showing the fluorescence intensities for binding with fluorescently tagged lectins ConA, WGA, RCA, ECA, PSA, and LCA with the chemo-enzymatically prepared glycan array.

larger structure.[36] Binding constants for a large number of PA conjugate glycans have been determined by frontal affinity chromatography[37] and more recently with the help of glycan arrays.

The latter format allows the high-throughput analysis of thousands of carbohydrate−lectin interactions on a single slide as reports involving the Consortium of Functional Glycomics array and other approaches have impressively demonstrated.[38] To our

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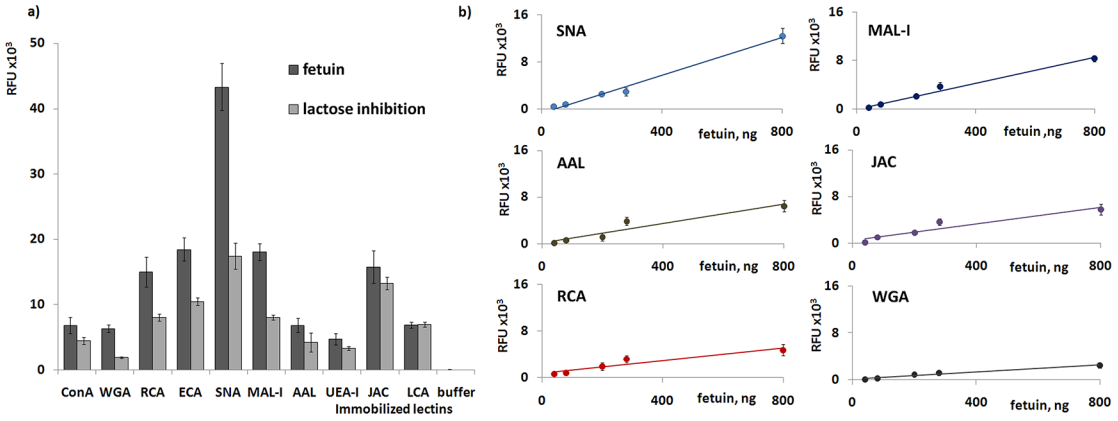

Figure 3. Specificity and linearity of the lectin array blot. (a) Transfer of a single protein to an array of 10 lectins; inhibition of carbohydrate−lectin interaction with lactose affects primarily lectins RCA, ECA, SNA, and MAL-I. (b) Linearity of fetuin transfer and trapping demonstrated for 6 different lectins.

knowledge, however, a systematic evaluation of the plant lectin specificities toward *N*-glycan structures, which would possibly permit a more detailed interpretation of the binding profiles, has not been published so far.

To this end we prepared a glycan array presenting a variety of mammalian *N*-glycans and some smaller epitopes[39] found on glycoproteins, with a systematic variation of the number of antennas, type of branching, terminal sugars, and core modifications, and evaluated their binding to the 20 lectins used in our lectin array blotting method. For the construction of the array, 24 synthetic glycans comprising complex, hybrid, and high mannose glycans and some smaller epitopes were spotted onto glass slides, and the structures were diversified into an array of 76 individual glycans by on-chip incubation with recombinant glycosyltransferases as previously reported.[40,41] We focused our binding studies on the interaction of plant lectins with *N*-glycan arrays, which until recently have received less attention due to their challenging synthesis and isolation, while lectin specificities toward *O*-glycans also present on many glycoproteins have been the subject of previous binding studies.[38]

The binding profiles obtained after screening this array with the panel of 20 lectins followed the known monosaccharide binding specificities reported in the literature, which are summarized in Table S-1 (Supporting Information). In addition, the screening allowed valuable insights into the change of affinity within closely related structures and some examples for surprisingly promiscuous lectin binding (Figure 2). These include the interactions of complex biantennary glycans with mannose-specific lectin ConA independently of the terminal sugar, the PSA binding to non-fucosylated pauci-mannosidic structures **3** and **4**, or the binding of the fucose binding lectin LCA to non-fucosylated complex and hybrid structures. The α-1,6 fucosylated biantennary structure **48** had been identified by the group of Hirabayashi as the preferred ligand for LCA among the tested PA-derived naturally isolated glycans.[42] We have found that structures lacking a second GlcNAc residue on the 6-arm such as the hybrid type core fucosylated *N*-glycans **53**−**56** show by over 2-fold enhanced binding to LCA compared to that of **48**. Finally, of all mannosylated structures lacking the single GlcNAc on the 3-arm only the trimannoside **24** containing α-1,2 linked mannose residues is bound. A single GlcNAc on the 3-mannose arm or α-1,2 linked mannose residues seem to be required for LCA binding at least for the structures present on our array.

Other notable results of this study are the reduced binding of WGA to complex structures after sialylation and fucosylation or the residual binding of RCA to sialylated structures **70** and **71** for which no binding to ECA was observed. ECA and RCA binding to galactosylated structures generally increased with the number of terminal galactose residues, but in addition some interesting effects of the type of branching or the presence of α-1,6-core fucose can be seen, which cannot be generalized due to the limited number of examples available.

The broad or unclear binding specificities of some lectins can compromise their utility for the detection of specific structural epitopes within complex glycoprotein mixtures, and the large differences in binding strengths for different lectins have to be taken into account in the probe selection for lectin arrays and generally other multilectin applications. The lectin binding data obtained from glycan array and frontal affinity experiments nevertheless support the use of many lectins as specific binding probes for the detection and identification of glycan epitopes of varying size and complexity in general profiling methods. Still, further lectin binding studies on large glycan arrays or defined glycoprotein probes on lectin arrays are needed to better understand the specificities of the growing number of plant and animal lectins available as carbohydrate-specific probes.

In a proof of principle experiment we transferred RNase B, glycosylated with high mannose N-glycans structures,[43] and BSA, as negative control, by diffusion-blotting[35] to a glass slide covered with over 23,000 printed spots (130 dpi) of mannose-specific Concanavalin A (ConA) (Figure 1c). Homogenous RNase B trapping with very low lateral diffusion was observed, while BSA was not transferred. Transfer of RNase B from the gel could be observed down to 1 ng of loaded glycoprotein, and a linear dose response was found until 10 ng of loaded glycoprotein. At higher concentrations saturation occurred (see Supporting Information). The general procedure was then optimized for selective carbohydrate lectin binding by adjusting pressure, blotting time, temperature, buffer compositions, protein fixing conditions, and lectin printing concentrations, and finally a method showing low assay interference of nonglycosylated proteins like serum albumins even at high concentration was developed.

Next we investigated the analysis of fetuin, a heavily *N*- and *O*-glycosylated 48.2 kDa glycoprotein, by trapping on slides covered with multiple arrays of 10 lectins specific for major carbohydrate epitopes comprising *Concanavalin A*, (ConA), wheat germ agglutinin (WGA), *Ricinus communis* agglutinin

dx.doi.org/10.1021/cb300262x | *ACS Chem. Biol.* 2012, 7, 1729−1737

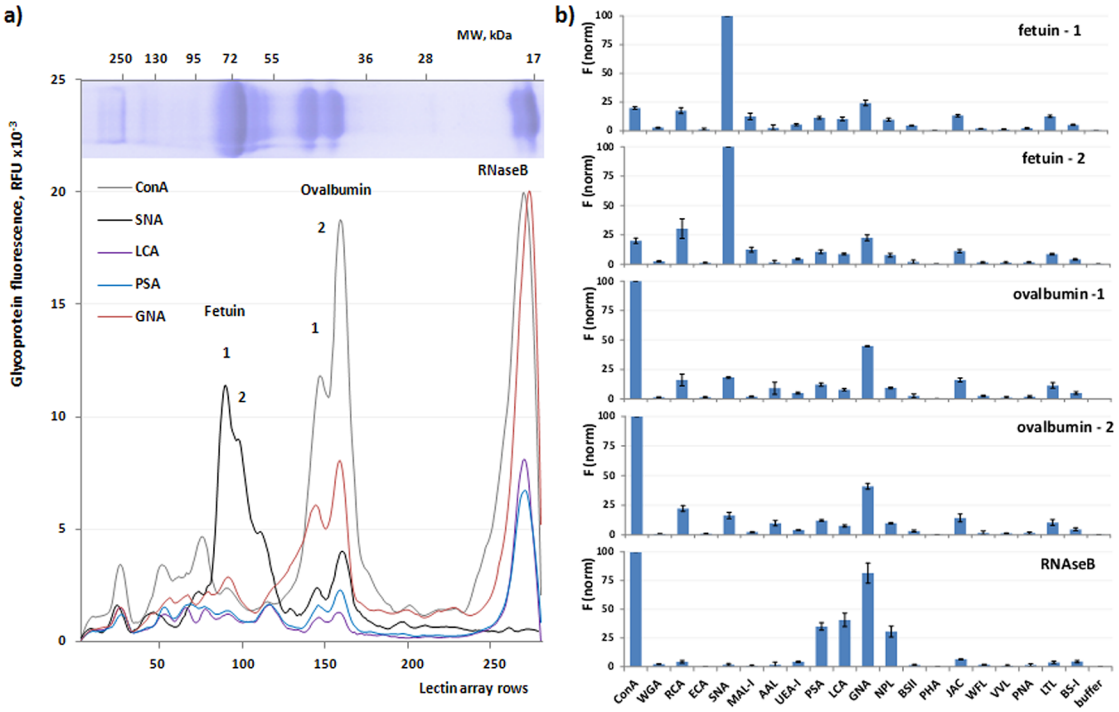

Figure 4. Glycoanalysis of an artificial mixture of 4 proteins by lectin-array blotting to the 20-lectin array. (a) Superimposed interaction profiles of separated proteins with 5 selected lectins after lectin array blotting, and Coomassie stain of SDS-PAGE gel with fractionated RNase B, ovalbumin, fetuin, and BSA. (b) Interaction profiles for 5 protein bands with the panel of 20 lectins included in the arrays.

(RCA), *Erythrina cristagalli* lectin (ECA), *Sambucus nigra* agglutinin (SNA), *Maackia amurensis* lectin (MAL-1), *Aleuria aurantia* lectin (AAL), *Ulex europeus* agglutinin (UEA), Jacalin lectin (JAC), *Lens culinaris* agglutinin (LCA), and control buffer. Lectins were printed at concentrations that had been adjusted in test blots with the artificial glycoprotein mixture to minimize nonspecific binding and at the same time ensure sufficient signal intensity for all specific interactions.

The binding profile for fetuin reflects well the reported presence of complex bi- and triantennary oligosaccharides (SNA, ECA, RCA, ConA) and *O*-glycosylation (JAC), while AAL binding indicated the presence of fucosylated structures (Figure 3a). Addition of lactose to the transfer buffer, which is known to inhibit RCA, ECA, SNA, and MAL-1, notably reduced the interaction with these lectins, demonstrating the selectivity of the glycan−lectin interaction (Figure 3a).

Moreover, a linear dose response was established for all fetuin-binding lectins over a concentration range of 40−800 ng (Figure 3b). An artificial mixture of RNase B, fetuin, ovalbumin, and BSA was then analyzed by diffusion blotting to a slide with arrays of 20 lectins, providing a more differentiated picture of protein glycosylation. To the 10 previous lectins were added *Galanthus nivalis* agglutinin (GNA) and *Narcissus pseudonarcissus* lectin (NPL), *Pisum sativum* (PSA) and *Lotus tetragonolobus* (LTL), *Griffonia simplicifolia* (BS-II), *Phaseolus vulgaris* (PHA-E/L), *Wisteria floribunda* agglutinin (WFL), *Peanut agglutinin* (PNA), *Vicia villosa* lectin (VVL-B4), and *Griffonia simplicifolia* (BS-I). Figure 4 shows the interaction profiles of 5 resolved gel bands with the 20 lectins.

For RNase B major interactions were observed as expected only with mannose-binding lectins ConA, PSA, LCA, GNA, and NPL. Our glycan array data seem to indicate that LCA shows preference for structures containing the α-1,2-dimannoside epitope present in many high mannose structures (Figure 2). Ovalbumin, a 45 kDa glycoprotein predominantly decorated with smaller high-mannose N-glycan structures (M5, M6), was separated into two known isoforms,[44] both showing major interaction with mannose-binding lectins ConA and GNA. The two fetuin bands in turn showed a dominant interaction with SNA, distinctive of a high degree of α-2,6 sialylation and less strong interactions with ConA, RCA, GNA, MAL 1, PSA, LCA NPL, LTL, or Jacalin typically of complex fucosylated glycans.

The method showed good reproducibility in a study quantifying three lectin array blots of the artificial protein mixture used previously. Two blots were taken side by side on two channels of the same gel, and a third blot was performed completely independently on a different day. After normalization to the lectin with the highest fluorescence, the main bands for fetuin, ovalbumin, and RNase B were analyzed. The data from the three experiments were collected and compared (see Supporting Information). The coefficients of variation (CVs) of the lectin interactions were 3−9% (n = 3) for RNase B, 6−18% (n = 3) for fetuin heavy band, and 10−19% (n = 3) for ovalbumin. Note that the bands corresponding to fetuin and ovalbumin overlapped, leading to higher errors than completely separated glycoproteins as RNase B.

Next we fractionated a human urine sample by SDS-PAGE and analyzed protein glycosylation in the sample with a slide containing the previous 20 lectins. Electrophoretic methods have been employed for the search of urinary protein markers for several pathologies including renal disease and cancers of the urogenital tract,[45] and the ability to analyze their glycosylation pattern could help to increase their prognostic value. Tamm-Horsfall protein (THP), the most abundant urinary glycoprotein, was detected by Western blotting using anti-THP as two bands with an electrophoretic mobility near 70 kDa. Seven bands were selected for lectin analysis, based on major interactions with the lectins RCA and LCA (Figure 5).

dx.doi.org/10.1021/cb300262x | *ACS Chem. Biol.* 2012, 7, 1729−1737

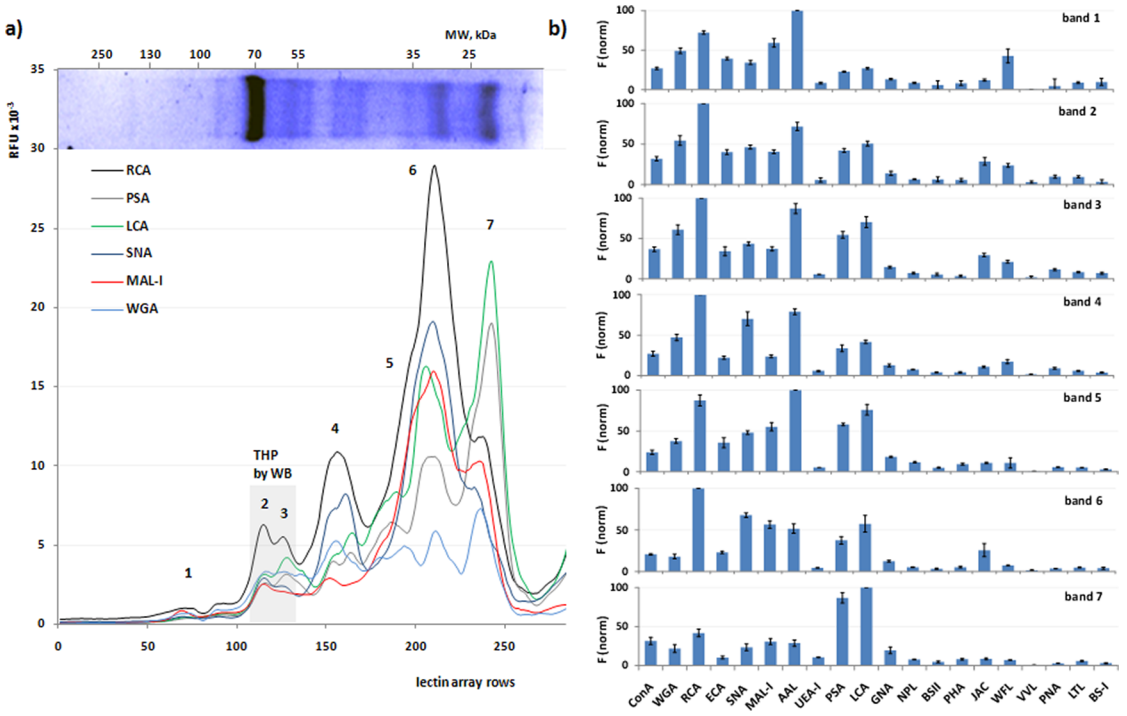

Figure 5. Glycosylation analysis of urinary proteins by lectin-array blotting to 20-lectin array. (a) Superimposed interaction profiles of 6 lectins with fractionated urinary proteins, and Coomassie stain of SDS-PAGE of urinary proteins. (b) Histograms showing interaction profiles of 7 selected protein bands with the 20-lectin array; for gel band numbering see profiles in panel a.

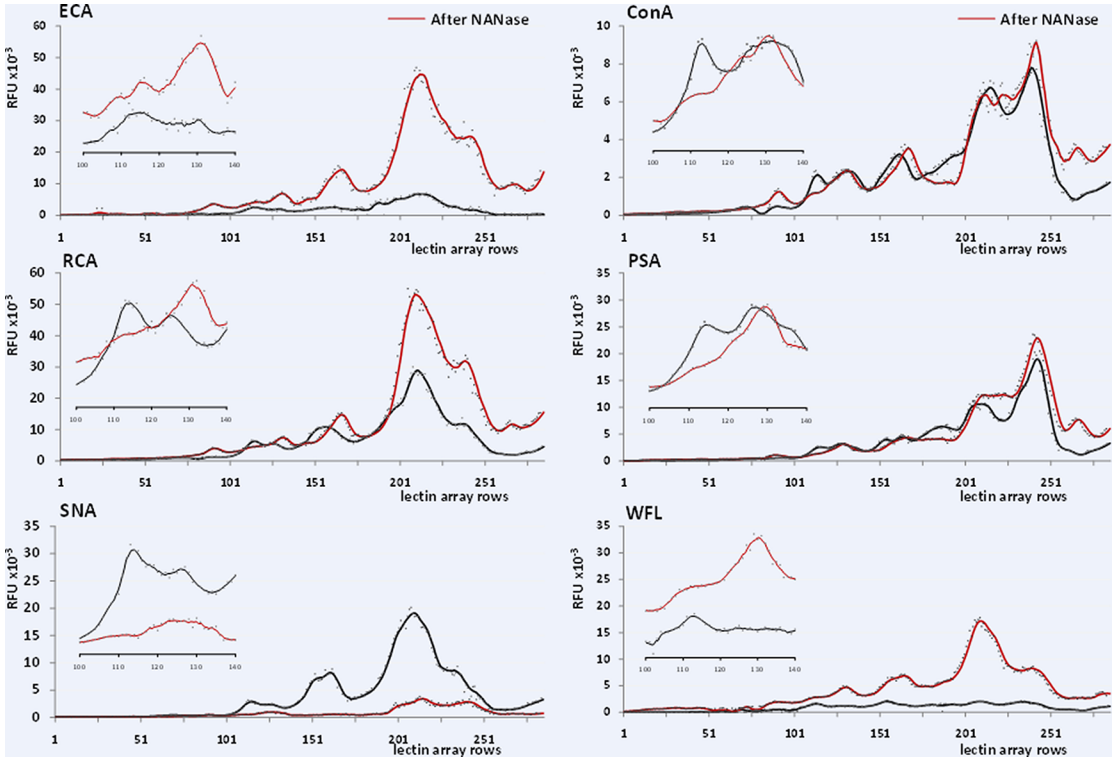

Figure 6. Lectin binding profiles of the urinary protein sample before and after neuraminidase treatment (black lines: urinary proteins profiles; red lines: profiles after neuraminidase (NANase) treatment; dotted line: raw data).

The two bands assigned to THP (bands 2−3 indicated in Figure 5a) showed a similar overall binding profile for *N*-glycosylation, suggesting the presence of a mix of high-mannose (ConA) and complex sialylated (MAL-I, SNA) and fucosylated structures (AAL).

Other far less intense gel bands (bands 1, 4, 5, and 6, Figure 5a) presenting minor components of the protein mixture were analyzed in a similar fashion and showed characteristic individual interaction profiles with the panel of 20 lectins (Figure 5b), which would have been probably covered up by the major

dx.doi.org/10.1021/cb300262x | *ACS Chem. Biol.* 2012, 7, 1729−1737

protein components in a traditional lectin array of unfractionated proteins (Supporting Information Figure S-21).

Where the resolution of 1-dimensional SDS-PAGE for the separation of protein mixtures is sufficient, individual lectin binding profiles can be recorded for every protein component (Figure 4). Depending on the protein concentration, an average gel band will cover around 15−40 lines on the array. For the histogram presentation (Figure 5b) 5−6 array lines at the peak maximum were quantified and normalized to the lectin showing the highest fluorescence value. This normalization is avoided in the profile representation (Figure 5a), which shows absolute fluorescence values for the lectin specific interaction profile with proteins along the gel slab.

For very complex mixtures, especially glycoproteins that can show significant band trailing in SDS-PAGE due to differently charged (sialylated) glycoforms, a fingerprint profile over the entire gel will be obtained rather than a glycan analysis of single proteins (Figure 5a).

To resolve single glycoforms and generally separate complex protein mixtures into single entities 2D SDS-PAGE is often employed,[46] and lectin array blotting for the analysis of individual glycoproteins should in principle be applicable.

In this case the gel serves well as a fractionation device with very reproducible performance allowing valuable insight into the differences in glycosylation for protein fractions at a resolution of over 285 lines per slide.

The lectin profiles were in good agreement with the data obtained in the lectin array analysis of the whole urinary glycome (against the 20 lectins immobilized in wells format) and with the lectin blots with labeled lectins in a nitrocellulose membrane after electrophoretic fractionation (see Supporting Information).

A major application of glycan profiling methods is the rapid detection of relevant glycosylation changes between disease and control groups, providing leads for future diagnostic markers. Encouraged by the good reproducibility of our method, we compared the lectin binding profiles of a urinary protein sample before and after treatment with a neuraminidase from *C. perfringens*, which cleaves α-2,3-, α-2,6-, and α-2,8 linked sialic acid. The urinary protein sample was labeled previous to the treatment with the neuraminidase to make the hydrolase invisible during the transfer to the lectin array. The interaction profile with 6 of the 20 lectins used in the blot before and after enzymatic desialylation shows as expected that interaction with lectins recognizing terminal sialic acid such as SNA, MAL-1, or WGA was reduced to background levels (Figure 6). Other lectins such as ECA and WFL showed a notable increase in binding, reflecting improved accessibility of newly exposed galactose and *N*-acetylgalactosamine residues after desialylation, while RCA showed a notable shift toward lower masses, which could be explained by a change of the electrophoretic mobility for partially sialylated glycans after complete desialylation. The difference in ECA and RCA binding profiles might be explained by the presence of fucosylated, non-sialylated structures that are picked up by ECA with exceptionally strong affinity as shown by our glycan array data (Figure 2).

An enlarged detail of the region containing the corresponding bands of the Tamm Horsfall protein, which was detected by Western blot, provides a more precise view of the effect of desialylation on the binding profile of a single protein or gel band (Figure 6). As removal of sialic acid can have a pronounced effect on the electrophoretic mobility of glycoproteins, gel bands for THP 2 and 3 (Figure 5a) were merged into a single band after neuraminidase treatment.

Conclusion. We could show that the combination of protein fractionation by gel electrophoresis together with a diffusion transfer to lectin-functionalized glass slides provides a fast and simple method to simultaneously analyze both *N*-and *O*-glycosylation in complex mixtures. Our method produces the equivalent of 20 lectin blots in a single experiment, giving detailed insight into the binding epitopes present in the fractionated proteins. As such this method can serve as a first qualitative attempt to analyze the glycome of an organism with a selection of 20 or more lectins, which can be followed up by more sophisticated mass spectrometry techniques for the structural identification of glycan biomarkers.[47] In contrast to other related methods such as antibody-overlay or antibody sandwich arrays, which are powerful approaches for diagnostic arrays, lectin array blotting can visualize protein glycosylation of all present proteins and is not limited to a predefined subset. Current limitations of our method are the number of lectins that can be printed in a single array and more generally the resolution of one-dimensional SDS-PAGE. In addition, lectin binding studies with synthetic glycan arrays have shown that some lectins bind to multiple epitopes, which can compromise their use as specific probes and highlights the need for a careful selection of lectins for the array to maximize the structural information obtained.

The importance of our method as an array-based glycoproteomics tool lies in its potential as a discovery tool for exploring protein glycosylation in an unbiased fashion and more rapidly than previously achieved by conventional lectin blotting or mass spectrometric approaches. It fits neatly in the classical proteomics workflow, and the identification of potential biomarkers by in-gel digestion and subsequent mass spectrometric analysis of selected gel-bands is possible as the major protein content remains in the gel after diffusion blotting. The development of high-throughput lectin array blotting and its application to non-hypothesis-driven glycan biomarker research is currently underway in our laboratory.

METHODS

Microarray Fabrication. Lyophilized lectins were reconstituted in HEPES buffer (50 mM, pH 8.5, 0.3 mM Ca^{2+}, 0.08% NaN_3), and stock solutions (2.0−5.0 mg mL^{-1}) were stored at −20 °C. Print solutions of lectins (0.4−0.5 mg mL^{-1}, in PBS, 0.01% of Cy3-conjugated BSA, 1.0 mM D-glucose) were prepared freshly prior to every print run from stock solutions. Source plates for printing containing 20 μL aliquots of 1, 10, or 20 lectins and control buffer were prepared in 384-well plates. Microarrays were spotted at 50% humidity (0.33 nL volumes, 0.2 μm pitch) according to one of 3 designs (see Supporting Information for details) onto NHS-activated glass slides (NexterionH, Schott AG) by a noncontact piezo-electric printing robot (Piezoarray, Perkin-Elmer). **Lection array design v1.0:** Single lectin array (WGA or ConA), 99 × 240 grid, 23760 spots. **Lectin array v2.0:** 10 lectins ConA, WGA, RCA, ECA, SNA, MAL-I, AAL, UEA-I, LCA, JAC and buffer, 11 × 1 subarrays, subarray grid 10 × 225, 22275 spots. **Lectin array v3.0:** 20 lectins ConA, WGA, RCA, ECA, SNA, MAL-I, AAL, UEA-I, PSA, LCA, GNA, NPL, BS-II, PHA, JAC, WFL, PNA, VVL, LTL, BS-I, and buffer, 21 × 1 subarrays, subarray grid 4 × 285, 23940 spots. Printed slides were incubated at 75% humidity, 18 °C overnight and stored at −20 °C without quenching if not used immediately. For immediate use, the unreacted NHS groups were quenched for 1 h with a 50 mM ethanolamine solution in borate buffer, washed with a 0.3 mg mL^{-1} BSA, 0.3 mM Ca^{2+} solution in PBST0.05 for 1 h and dried by centrifugation.

SDS-PAGE Electrophoresis. Calf serum fetuin, chicken albumin, bovine ribonuclease B, and IgG free BSA, (Sigma Aldrich) were prepared as 2.0 mg mL^{-1} total protein solutions in PBS, diluted 1:2 with phosphate buffer, pH 8.5 and labeled with Hilyte Plus 647 (Cy5 analogue) labeling kit (AnaSpec). Labeled glycoproteins were stored as 1−2 mg mL^{-1} solutions in PBS at 4 °C. Labeled glycoproteins were

treated with Laemmli buffer, and 10 μL containing 0.1−1 μg of protein (mixture) was loaded onto the SDS-PAGE gel (12% polyacrylamide, 1.0 mm). Electrophoresis was carried out in TGS buffer at 150 V for 1 h at 4 °C. Silver and Coomassie stains were performed according to standard procedures, and gel images were recorded on a VersaDoc imaging system from Bio-Rad.

Lectin Array Blot. After electrophoresis the gel was treated with 20 mL of fixing buffer (20% MeOH in H_2O (v/v)) for 30 min, washed with PBS (1 min) and blocking solution (0.3 mg mL^{-1} BSA, 0.3 mM Ca^{2+} solution in PBST0.05) for 3 min, placed on a filter paper, and air-dried for 30 min. A lectin array slide was placed on top of the gel in such a manner that the protein ladder was completely covered by the lectin-functionalized area, and gel and slide were aligned in the direction of electrophoresis. This filter paper−gel−slide sandwich (see Figure 1) was placed between two glass plates, pressed with a 1 kg weight, and incubated in a prewarmed plastic container at 37 °C for 60 min at ca. 90% humidity. Slide and gel were separated with tweezers, and the slide was washed with PBS for 5 min under gentle rocking. After drying in a slide centrifuge, the slide was ready for scanning. As only a fraction of the glycoproteins are transferred, the gel can be used for a second blotting, additional staining, or in-gel protein digest and subsequent mass spectrometric identification of proteins in specific bands.

Image Analysis. Fluorescence measurements were performed on an Agilent G265BA microarray scanner, Agilent Technologies, image quantification was performed with ProScanArray Express software from Perkin-Elmer, and interaction profiles with the lectin array were displayed as histograms using Microsoft Excel 2007. The adaptive circle method with a diameter range of 65−70 μm was employed for spot quantification. The median value was used for the fluorescence of each spot, and for every lectin the average of 5 replicate spots was used to construct histograms showing the binding profile. Intensity values in the histograms were normalized to the highest signal. Error bars are included showing the standard deviation for each lectin/protein interaction.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures for method optimization and validation and full glycan binding data. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): We declare that we have filed an international patent application related to the presented method.

■ ACKNOWLEDGMENTS

We are very grateful for the recombinant cell lines expressing two glycosyltransferases used in this study that were provided by the laboratories of J. Paulson, Scripps Research Institute, San Diego and I. Wilson, BOKU University, Vienna. Funding from Ministerio de Ciencia e Innovación, CTQ2008-04444/BQA grant, Government of the Basque Country, Etortek grant, the European Union, ITN-Euroglycoarrays grant, and a grant from the Guipuzcoan program of Science, Technology and Innovation, Guipuzcoan Regional Government is gratefully acknowledged.

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