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Solid-phase glycan isolation for glycomics analysis

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Abstract

Glycosylation is one of the most significant protein PTMs. The biological activities of proteins are dramatically changed by the glycans associated with them. Thus, structural analysis of the glycans of glycoproteins in complex biological or clinical samples is critical in correlation with the functions of glycans with diseases. Profiling of glycans by HPLC-MS is a commonly used technique in analyzing glycan structures and quantifying their relative abundance in different biological systems. Methods relied on MS require isolation of glycans from negligible salts and other contaminant ions since salts and ions may interfere with the glycans, resulting in poor glycan ionization. To accomplish those objectives, glycan isolation and clean-up methods including SPE, liquid-phase extraction, chromatography, and electrophoresis have been developed. Traditionally, glycans are isolated from proteins or peptides using a combination of hydrophobic and hydrophilic columns: proteins and peptides remain on hydrophobic absorbent while glycans, salts, and other hydrophilic reagents are collected as flowthrough. The glycans in the flowthrough are then purified through graphite-activated carbon column by hydrophilic interaction LC. Yet, the drawback in these affinity-based approaches is nonspecific binding. As a result, chemical methods by hydrazide or oxime have been developed for solid-phase isolation of glycans with high specificity and yield. Combined with high-resolution MS, specific glycan isolation techniques provide tremendous potentials as useful tools for glycomics analysis.

Keywords

Dynamic covalent chemistry; Hydrazide; Immobilization; MALDI-TOF MS; Solid-phase extraction

1 Introduction

Glycomics has been a critical facet of postgenome science since glycans ubiquitously modify a great number of proteins (Fig. 1). Glycoproteins play key roles in many physiological events such as signal transduction, inflammation, tumorigenesis, cell development, and differentiation [1]. It has been well recognized that glycoprotein analysis is an important field of interest especially for diagnostics, prognostics, or therapeutic targets since the majority of human membrane and secreted proteins is glycosylated [2,3]. Numerous diseases have been attributed to upregulation or downregulation on one or several glycoproteins including coronary heart disease [4], congenital disorders of glycosylation [5], Tn syndrome [6], inflammatory bowel disease [7], prostate cancer [8], and ovarian cancer [9]. Additionally, glycoproteins modified with different glycans may exhibit different biological functions [10]. Alteration of glycans could dramatically change glycoprotein

functions; thus, it is intrinsically related to the disease development [8]. For example, glycosylated proteins are released from malignant cells carrying disease-related carbohydrate epitopes into the interstitial space where glycoproteins further reach the circulation (Fig. 1). As a result, glycans are particularly interesting targets that offer a few major advantages over proteins, including biological design, amplification effect and stability [11].

In general, glycan analysis starts with glycoprotein extraction from tissues, cells, or body fluids followed by glycan isolation, clean-up and/or separation, and detection. To study glycoproteins, glycans and their glycosylation sites, glycoproteins are enriched and their glycans are released by an enzyme or chemical reaction before digestion by trypsin [12]. Both formerly glycosylated peptides and released glycans are analyzed for identification of glycoproteins, glycosylation sites, and glycan structures by techniques such as ESI-MS or MALDI-MS. Currently, several strategies including chemical immobilization and affinity capture of glycans using lectin reagents have been developed to study glycoproteins [12–17]. These techniques have been successfully applied to quantitative glycoproteomics using MS in a variety of diseases [18–20]. However, isolation of glycans from complex mixtures with high specificity using chemical approaches remains to be developed. We reviewed current glycomics technologies for glycan purification, isolation, separation, and detection.

2 Glycan purification

Due to hydrophilic nature of glycans, analysis by MS becomes challenging when the glycans are analyzed in the mixture of hydrophobic proteins and peptides that are more efficiently ionized and suppress the ionization of glycans. Purification of glycans from complex mixtures of salts, detergents, proteins, and peptides prior to their detection by MS is critical because salts and detergents further interfere with the ionization of glycans by MS analysis.

Glycans for MS analysis are generally prepared in several steps. After glycoproteins are isolated, they are either directly analyzed, or digested to peptides. Then, the glycoproteins or glycopeptides are enzymatically treated to release *N*-linked glycans or chemically treated to obtain *O*-linked glycans. Third, the glycans are purified from the rest of the mixture consisting of peptides, other enzymes or chemical reagents used in the glycan releasing reaction. Methods to effectively separate or isolate glycans from the mixture are crucial for identification of glycans by MS.

One of the common techniques for glycan and peptide separation is to use hydrophilic interaction liquid chromatography (HILIC) [21–23]. Zwitterionic type of HILIC column demonstrates reasonable performance on separation of glycans, glycopeptides, and nonglycopeptides by recognition of isomeric glycans. The separation is mainly based on different combination of hydrophilicity and hydrophobicity of glycans and peptides [24]. However, without derivatization of glycans, the retention of glycans on HILIC absorbent is usually maintained low, so that some glycans can be lost in the flow-through and significantly decrease glycan recovery. Many researchers took advantage of graphitized carbon columns as an RP medium to purification and separation of unlabeled glycans for MS analysis [25–27]. We investigated the separation efficiency of glycan-peptide isolation using graphitized carbon column for mixture of glycans, peptides, and salts. Results clearly indicated that glycans elute in a lower concentration of ACN while peptides tend to elute at a higher concentration. However, in an intermediate ACN concentration, both glycans and peptides were eluted simultaneously [28]. These findings show that single dimensional (1D) chromatography is insufficient for glycan isolation.

Due to the fact that the unmodified glycans have little or no retention on hydrophobic absorbent, such as on C-8 and C-18, glycans in the protein/peptide mixture can be isolated using 2D chromatography or electrophoresis [29, 30]. For example, glycans were purified upon release from glycoprotein using 2D chromatography consisting of a C-8 column integrated with a graphitized carbon column [31]. Using the 2D procedure, peptides are retained on the C-8 column while glycans and other hydrophilic substances are collected in flow-through solution. Glycans are then enriched on a graphitized carbon column by hydrophobic interaction while salts and other hydrophilic molecules in solution are washed away in flow-through. Such sample clean-up method is straightforward and promising for development of a microfluidic platform in which hydrophobic resins are packed in the first channel and the graphitized resins are packed in the second channel. Cleanup can be achieved by passing sample through both channels in sequence, where the first channel is intersected with the second channel.

The potential concerns on these chromatographic methods are binding capacity between analytes and absorbent since glycans and nonglycan molecules can compete with each other by interaction with the absorbents. Thus, glycan purification performance is largely dependent on their differences on binding capacity. Although glycans are usually hydrophilic, their properties can vary with respect to their carbohydrate compositions and size of glycans. On the other hand, peptides have a wide range of hydrophobicity when different side chains exist [32, 33]. As it was observed, low molecular peptides were co-eluted with glycans, affecting the glycan identification by MS [34,35]. In addition, some glycans were even weakly bound on C8 or C18 absorbents, enabling the glycans to be lost, further affecting the overall yield of glycan recovery. Nevertheless, multidimensional microfluidic platform-based chromatographic technique could be a promising approach to replace traditional chromatographic separation in increasing the glycan recovery.

3 Dynamic covalent chemistry for glycan isolation

Dynamic covalent chemistry (DCC) is a chemical reaction that can be carried out in a reversible manner under certain conditions and equilibrium control [36]. Targeted molecules are conjugated and released via reversible bond formation, so that DCC has a great potential in fields of drug discovery and biology [37–40]. Glycans have single reducing end after they are released by either PNGase F or β -elimination. The reducing end co-exists in both a cyclic and an acyclic form, with the latter structure involving an aldehyde group that can conjugate with hydrazide groups [41]. At a neutral pH, the majority of the glycans are in the cyclic form that minimizes hydrazone formation. By addition of aniline, the acyclic structure dominates the glycan reducing end and tremendously increases glycan conjugation to the hydrazide group because aniline reacts to reducing ends and forms aniline Schiff base intermediates [38, 42]. Even without being reduced, hydrazone bond is stable in weak base condition for its relatively low hydrolysis rate [43]. Further, decrease of buffer pH can significantly increase hydrazone hydrolysis and completely release glycans (Fig. 2). Thus, it allows capture of glycans to hydrazide resin in mild acid solution while peptides, detergent, and other reagents are removed using base buffer.

The DCC was applied for glycan isolation in complex sample mixture [28, 44]. In this method, glycans are released from denatured glycoproteins or trypsin digested glycopeptides. Then, the released glycans are conjugated to a solid support resin containing hydrazide groups via the glycan reducing ends. The glycan conjugated resin is further washed to remove nonspecific binding species followed by incubation of the glycan conjugated resin under acidic conditions (pH < 3.0) to release the glycans by hydrazone hydrolysis. Alternatively, glycans could be hydrolyzed from resins via competition reaction using high concentration of formaldehyde (e.g., 10 $\times$ over glycan concentration) to minimize

loss of sialic acid in acid solution [45] or stabilize sialic acid by amidation [46] and esterification [47]. The released glycans are then collected and analyzed by MS. The procedure is briefly illustrated in Fig. 3.

To develop the solid-phase glycan capture method, we used a set of standard glycans to determine conditions for glycan conjugation and hydrolysis. It has been concluded that conjugation has been performed in optimum conditions when pH ranges from 4 to 6 [48, 49]. In neutral pH, glycan conjugation is less than 10% by estimation of the intensity ratio between glycan and its conjugation products in MALDI-MS detection. Glycan-hydrazide conjugation is impressively increased up to approximately 90% at pH 5.0 in the presence of aniline. Without addition of aniline, less than 50% conjugation can be achieved at pH 5.0. The glycan-hydrazide conjugation is completely hydrolyzed in 10% formic acid. It should be noted that hydrazone is reversible, but its conjugation and dissociation rates are not identical under different pH and reagent conditions [43]. For example, conjugation of sulfonic acid hydrazide and benzaldehyde at pH 2.47 with the rate constant for hydrazone formation is approximately $0.76 \times 10^{-4} \text{ s}^{-1}$ and hydrolysis constant is $0.36 \times 10^{-4} \text{ s}^{-1}$. At pH 4.48, formation constant drops to $0.20 \times 10^{-4} \text{ s}^{-1}$ while hydrolysis constant drops further to $0.08 \times 10^{-4} \text{ s}^{-1}$. When the conjugation is conducted on solid phase, hydrolysis can thus be readily controlled by pH. The conjugated hydrazide glycan can be maintained under weak base buffer conditions for the removal of nonglycan material and then the hydrazone can be hydrolyzed under acid conditions for recovery of glycans. In addition, use of aniline can facilitate formation of aldehyde at the reducing end while minimizing ring structure formation with hydroxyl in C5 [38]. Comparatively, solid-phase conjugation has great advantages over solution based reaction, since buffer can be easily exchanged on solid support resins. The glycan loss due to hydrolysis can be minimized since quick pH change can be performed and much lower hydrolysis is expected at a higher pH condition. For instance, half-life of aliphatic aldehyde-based hydrazone conjugation is longer than 120 h at pH 7.4 while half-life of same compound drops to shorter than 2 h at pH 5.5 [50].

The reversible hydrazone solid-phase glycan extraction (rHSPE) was applied for isolation of glycans from human serum sample. We mixed 2 μL of serum with 3 μL of 10 mM standard glycans (DP4: maltotetraose; DP5: maltopentose; DP6: Maltohexanose; DP7: maltoheptaose). The mixture was denatured and digested by trypsin. After deglycosylation by PNGase F, the sample was used for glycan isolation by rHSPE. The results are shown in Fig. 4. Several conclusions were drawn by use of rHSPE. First, rHSPE is specific on glycan capture and all four spiked DPs were clearly observed after rHSPE capture. They were not detectable before glycan capture (Fig. 4). Second, rHSPE has the potential to extract glycans from complex biological samples. In this study, 17 *N*-linked glycans from serum were identified by MS after capture-release process using this method, as well as four standard glycans that were spiked to human serum. We expected that additional glycans were present in human serum samples, especially the complex glycans with terminal sialic acids. The derivatization of sialic acids by permethylation or esterification [46, 47] will facilitate the identification of sialylated glycans by MS. In addition, release of the glycans from hydrazide resins using less acidic conditions will preserve the sialylated glycans. Increased amount of serum samples can be also used to identify low abundant glycans.

Glycopeptides can also be captured on a solid support through oxime group [51] as shown in Fig. 5. Glycopeptides are first treated with galactose oxidase to produce an aldehyde, which can conjugate to a solid support on its oxime. Other nonconjugated substances in complex biological samples can be readily washed away for isolation and purification of the glycopeptides. More importantly, captured glycopeptides can be either cleaved by PNGase F to collect deglycosylated peptides, or chemoselectively reacted with the tag by transoximization. Both peptides and glycans can be released by incubation in 4 *N*HCl [52].

If glycoproteins are captured via oxidization of glycans with terminal galactose, other types of glycan are not applicable. Sialylated glycans would have to be desialylated to be digested with the galactose oxidase enzyme. Moreover, periodate oxidation of sialylated glycans can be used to conjugate glycoproteins by aniline-catalyzed oxime ligation [53]. Similarly, we can study sialylated glycoproteins using solid-phase approach using hydrazide chemistry [54].

Recently, hydrazide beads were also used to react with glycans and further label immobilized glycans via a transoximization approach [55]. Glycan mixtures obtained from enzyme-digested glycoproteins are first captured by beads functionalized with oxime groups. A fluorescent tag is used to release labeled glycans and label the reducing end of glycan by transoximization chemistry. The transoximization produces multiple tag exchange functions that are useful for glycan purification by immobilization. This technology may provide a powerful platform for construction of glycan microarrays where naturally derived glycans are used as other contents.

4 Solid-phase glycan capture

The key issue for enhanced glycan isolation by DCC is effective capturing on solid phase. Critical parameters for a solid-phase glycan capture are capture efficiency, covalent bond stability, and bond cleavability. The common glycans consist of several building blocks, including sialic acid, hexose (mannose, glucose, galactose etc), hexosamine (*N*-acetylglucosamine, *N*-acetylgalactosamine), deoxyhexose (fucose), pentose (xylose), and uronic acid (glucuronic acid and iduronic acid). All these monosaccharides have similar reducing ends. However, they have different functional groups such as carboxylic acid for sialic acid and uronic acid, amine for *N*-acetylhexosamine. The immobilization sites for glycans could be any of them. Method development for immobilization of glycan on solid phase via reducing end, amine or carboxylic acid is summarized in Fig. 6 and compared in Table 1.

Common method of capturing glycans is through their reducing ends as described previously since released glycans by enzyme or chemical reaction have single reducing end. Also, cleavable bonds formed at reducing ends could be potentially used to recover intact glycans. In weak acidic conditions, reducing ends tend to be arranged in acyclic form [38], creating aldehyde group for conjugation. Aldehydes can also conjugate with functional groups such as hydrazide (i), hydroxylamine (ii), cysteinamide (iii), and amine (iv, v), formation of hydrazone, oxime, thiazolidine, and imine, respectively [56, 57] (Fig. 6).

Hydrazide chemistry has been successfully demonstrated for capture and release of glycans via their reducing ends [55]. Immobilization of glycan reducing ends via an oxime bond has been studied by several groups [52, 58–61]. The conditions for the formation of the oxime are dimethylformamide and 0.1 M aqueous sodium acetate [62]. Glycans are dissolved in the solvent and incubated with the resin for 24 h at 40°C. Without further reduction, the immobilized glycans can be cleaved off the resin by incubation under acid solution (Fig. 6 (ii) c and d) e.g., 4 *N*-hydrochloride [52]. The oxime reversible linkage has several advantages over other linkers. First, the conjugation can be readily implemented under rather mild aqueous conditions since the oxime is more nucleophilic than hydrazide due to its α -effect. Second, oxime linkage is quite stable in a wide range of pH, providing stability for glycan purification and other substance removal at minimum sample loss. This strategy has been employed for glycoblotting glycans from crude proteolytic digest mixture [55]. The captured glycans can also be used for enzyme-linked immunosorbent assay detection, in which lactose and *N*-acetylchitooligosaccharides are anchored on a solid support via oxime formation at reducing ends [63].

Cysteinamide can also anchor glycan reducing end via its aldehyde group [62]. Pectin fractions in a water-ACN mixture at pH 4.0 are immobilized onto cysteine-terminated solid resins by formation of a thiazolidine linkage (Fig. 6 (iii)). The thiazolidine linker is stable under neutral and weak base conditions. Addition of potassium and acetic acid could release intact glycans from solid resins, recovery of purified glycans from SPE. Studies have shown that the dissociation rate of thiazolidine linkage was highly dependent on salt concentration and pH of the solution [64, 65]. The thiazolidine-based solid phase has been applied for synthesis of hydrogels as corneal adhesives for healing wounds [65]. Chitosan has been similarly immobilized on nonporous glass beads through thiazolidine linker and verified using NMR and infrared resonance [66].

The major disadvantage of using hydrazone, oxime, or thiazolidine is the instability of these structures under certain conditions. As a result, a few glycans immobilized on solid supports can be inevitably lost during multiple cleaning processes. A permanent covalent conjugation or permanent covalent chemistry may provide an ideal scenario to improving glycan capture efficiency and recovery yield. An example is to develop a bifunctional reagent (Fig. 6 iv) that contains a terminal amine at one end and a terminal disulfide at the other end. The reducing ends can be conjugated to the amine that is converted to a stable bond by further reduction step, similar to glycan fluorescence labeling by aminobenzamide [49,67] or aminobenzoic acid [22,68]. The disulfide terminal will enable immobilization onto a solid support, such as gold via self-assembled monolayer [69–71]. Glycans are then enriched on the solid support and released by cleaving disulfide bond via reduction. However, this approach modifies glycan-reducing ends by adding fixed molecular weight, resulting in complicated data analysis.

Other type of glycans e.g., glucosamine subunit, can be captured by a similar approach in which the amine on the glucosamine conjugates to the aldehyde groups on the backbone amide linker (BAL) solid-phase support [72, 73]. BAL has been applied to side-chain anchoring for peptides and glycans [72, 74]. Glucosamine is first immobilized onto the BAL solid support by the formation of the Schiff base and its subsequent reduction (Fig. 6 v). At a later stage, the conjugated glucosamines can be released from the solid support by treatment with TFA containing acetic anhydride and pyridine. By this approach, the released glycan is modified with acetic group increased molecular weight by CH_3CO^- (43 Da).

Many glycans also contain sialic acid, especially in a variety of disease sample from serum [75,76], tissues [77–79], and body fluids [80–82]. As an alternative approach, several labs developed an approach to specifically analyze sialylated glycans instead of analyzing global glycans in specimen, such as mannose and fucosylated glycans [83–85]. For instance, oxime reactive groups are anchored onto a solid support, and these groups can react with the oxidized sialic acid on sialylated glycans. Briefly, glycoproteins or glycopeptides are mixed with oxime beads at 37°C for 2 h after sialic acid is oxidized in 1 mM sodium periodate aqueous solution at 0°C for 15 min [54,83,85,86]. After removal of other substances, sialylated glycoproteins or glycopeptides can be remained on beads. Then, the sialylated glycopeptides are directly analyzed after hydrolysis of oxime linkage under acid condition; both formerly sialylated *N*-glycopeptides and their glycans can be studied by PNGase F treatment and/or oxime linkage hydrolysis.

5 Labeling glycans for structural and quantitative analyses

Isolated glycans at their native form or labeled at the reducing ends can be further analyzed by electrophoresis, chromatography, and MS. Many studies have demonstrated the separation of glycans using CE coupling with MS for qualitative and quantitation analysis [87–89]. More effectively, LC is applied for glycan profiling since it possesses high

separation resolution and a variety of separation media are available. The normal phase LC–MS can identify details of underivatized glycans in a few hours with a sensitivity that is in the femtomole range [90–92]. On the other hand, RP-LC has been developed for profiling and characterizing underivatized or labeled glycans by fluorescence detection and structural determination by MS/MS [25,31,93,94]. In many instances, underivatized glycans cannot be reliably and robustly identified by either chromatography or MS. Therefore, labeling and/or modification of glycans are an effective means widely used to increase glycan stability and hydrophobicity. For instance, glycan permethylation significantly enhances stability, facilitates absolute linkage identification by MS/MS [95, 96], and stabilizes the sialic acid residues [97]. Glycans are often labeled with a variety of reagents [97] including 2-aminopyridine [98], 2-aminobenzoic acid and 2-aminobenzamide [49], 2-aminonaphthalene trisulfonic acid [99], and 1-aminopyrene-3,6,8-trisulfonic acid [100]. Glycans that have been labeled by different fluorescence reagents showed considerable differences in detection sensitivity [101]. Thus, their detection enhancement in MALDI-TOF MS was also reported to be attributed to the labeling [102]. Since detailed discussion of labeling and MS is beyond scope of this review, further information on those topics can be found in numerous literatures.

6 Conclusions

In this review, we have summarized several technologies developed for glycan purification. Chromatographic techniques are simple and straightforward means to partially or completely purify glycans by 1D or 2D approach. Integration of C-18 or C-8 with graphitized carbon has been traditionally used for glycan preparation. Purification efficiency and recovery of glycan by chromatography could further be improved by minimizing the nonspecific binding of glycans. As an alternative approach, SPE of glycans presents itself as a reliable technique for glycan preparation since glycans can be efficiently isolated from other substances by dynamic or permanent covalent chemistry. Serum glycan isolation has been demonstrated using reversible hydrazone SPE of glycans. Other reversible linkers such as oxime, thiazolidine, and disulfide can be similarly applied for glycan isolation. Permanent covalent chemistry can significantly increase glycan capture and recovery although structures of the released glycans are modified at their reducing ends.

Glycan isolation by a solid-phase support can be applied in microfluidics. For example, packing of hydrazide beads in microfluidic can immobilize glycopeptides, followed by glycopeptides or glycans separation in the second channel via RP-LC. On-bead glycan permethylation can be performed online in microfluidics, offering several advantages over in-solution solid-phase methods including automation, easy interfacing with MS, simplified sample cleanup, minimized sample loss, and versatile detections. We expect that these technologies incorporated with the state-of-art MS will make a tremendous progress in understanding the protein glycosylation on disease progressions and identification of glycan biomarkers for the goal of disease prognosis and diagnosis.

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Abbreviations

BAL	backbone amide linker
DCC	dynamic covalent chemistry

HILIC	hydrophilic interaction liquid chromatography
rHSPE	reversible hydrazone solid-phase glycan extraction

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Figure 1.

Schematic illustration of protein glycosylation in malignant cells. The glycosylated proteins are released from malignant cells carrying disease-related carbohydrate epitopes into the interstitial space, whether glycoproteins further reach the circulation. Reprint and permission from [11].

Figure 2.

Schematic diagram of glycan capture and release on solid-phase via dynamic covalent chemistry. Glycans are catalyzed by addition of aniline to formation of Schiff-based intermediate and further reacted to hydrazide or amine. The hydrazone bond is stable at pH 7 or mild base condition, but can be easily hydrolyzed in acid condition e.g., 10% formic acid solution. Reprinted with permission from [28].

Complex biological samples

rHSPE: reversible Hydrazone Solid-Phase Glycan Extraction

Figure 3.

Schematic illustration of glycan capture procedure by reversible hydrazone solid-phase glycan extraction (rHSPE). Glycoproteins are first trypsin digested; glycans are then released by either PNGase F for *N*-linked glycans or β -elimination for *O*-linked glycans; glycans are conjugated to hydrazide beads; nonglycan molecules are removed by washing; conjugated glycans are finally hydrolyzed from solid phase either by acid hydrolysis or competition reaction by formaldehyde solution. Reprinted with permission from [28].

Figure 4.

Glycan isolation from human serum with addition of standard glycans and peptides by hydrazide beads. The bottom spectrum was the sample mixture without glycan isolation. The top spectrum was isolated glycans from hydrazide beads after conjugation, washing, and hydrolysis. Reprinted with permission from [28].

Figure 5. Solid-phase glycochemistry and transoximization for glycomics and glycoproteomics analyses. Digested glycopeptides are oxidized on antennary galactose; C-6 oxidized glycopeptides are conjugated with oxime to immobilize glycopeptides; glycopeptides are labeled with tag by transoximization reaction, or glycopeptides are released from solid-phase by PNGase F treatment. Reprinted with permission from [51].

Figure 6.

Methods for solid-phase capture and release of underivatized glycans. (i) Hydrazide chemistry for reducing end capture release. (A) pH 3–6 in methanol-acetic acid, microwave, 20 min. (B) 10% formic acid, or 200 mM formaldehyde at room temperature; (ii) Hydroxylamine chemistry for reducing end capture release. (C) DMF-water (1:1), pH 4.8, 40°C, 24 h. (D) 4N HCl hydrolysis; (iii) cysteamine chemistry for reducing end capture release. (E) ACN-water (1:2), pH 4.0, room temperature, 48 h. (F) KCL in DI, 50°C, pH <6, (acetate and acetic acid); (iv) disulfide chemistry for reducing end. (G) DMSO-acetic acid (7:3), 50 mM sodium cyanoborohydride, 65°C, 3 h. (H) Disulfide bond can be cleaved by 16 mM DTT, 60°C, 1 h; (v) Chemoselective reductive alkylation chemistry for amine glycans. (I) Sodium cyanoborohydride in DMF-acetic acid (99:1). (J) Glycan is released in acetic anhydride-pyridine, TFA-water (19:1) [72]. Reprinted with permission from [57].

Table 1

Summary of solid-phase methods for glycan isolation

Linkage	Isolation method	Reference	Application	Pros and cons
Hydrazide	Formation of hydrazone between glycan and hydrazide; release of glycans by hydrolysis	Yang et al. [28]	<i>N</i> -glycan isolation from serum	Pros: conjugation and hydrolysis of glycans can be controlled by pH; fast capture and release Cons: reversible hydrazone bonds reduce glycan recovery
		Miura et al. [103]	<i>O</i> -glycan enrichment from mucin	
		Lee et al. [104]	Carbohydrate microarray	
Hydroxylamine	Formation covalent bonding between oxime and reducing end of glycans; release of glycan by hydrolysis	Thygesen et al. [30]	Oxime functionalized AuNP for protein-carbohydrate interactions	Pros: ease of formation under mild aqueous conditions; high nucleophilicity via the α -effect; good stability under a wide range of pH; versatility in the choice of supports Cons: difficulty due to reduced hydrolysis compared to hydrazone bond
		Vila-Perello et al. [58]	Immobilization of oligosaccharide on specific protein-carbohydrate sensor surface	
		Dendane et al. [105]	Immobilization of oxime bond on to the inner wall of fused-silica capillary and on the surface of glass slides	
Cysteinamide	Anchoring of cysteine-terminated resins through formation of a thiazolidine linkage	Guillaumie et al. [57,62]	Immobilized amine-bond of cysteinamide on solid-phase and capture glycan via thiazolidine linkage	Pros: quantitative coupling; thiazolidine stability over a wide range of conditions Cons: coupling in multiple steps
Disulfide	Formation of disulfide bond on solid-phase support; release of glycan through cleavage of disulfide bond	Guillaumie et al. [57,106,107]	(GalA) _n functionalized sepharose supports; glycan reducing end conjugation with aminoxyacetamide	Pros: quantitative coupling of glycans; stable bond; glycan capture completion Cons: residue left on glycan reducing ends
Reductive alkylation	Formation of a chemoselective reductive alkylation of the 2-amino group; release of glycans by hydrolysis	Jensen et al. [72]; Rolborg et al. [108]	Unprotected <i>D</i> -glucosamine and glucosamine derivatives are attached to a trialkoxybenzyl linker on a solid support	Cons: quantitative coupling of glycans; good glycan capture yield; easy to release of glycans by hydrolysis at acid condition Pros: only suitable for glucosamine and its derivatives