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A General Method for Producing Bioaffinity MALDI Probes

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A bioaffinity probe based on the idea of immobilizing avidin on the probe surface to extract biotinylated oligosaccharide is described. The probe is produced by taking advantage of the natural affinity of proteins for hydrophobic polymer films. The avidin is immobilized by simply drying the solution on a polymer film surface. This produces a bioaffinity probe that shows enhanced activity for biotin-labeled oligosaccharides. The probe is produced in a matter of minutes but is highly effective for concentrating biotinylated oligosaccharide on the surface. The best matrix for the analysis is DHB, and the best film for the probe is a polyester material commonly used for transparency film. The efficacy of the probe is illustrated with neutral and anionic oligosaccharides. Oligosaccharides derivatized with biotin are retained while those that are unlabeled are washed away. No trace of the unlabeled oligosaccharide is observed in the mass spectrum.

The concept of biologically active probes for matrix-assisted laser desorption/ionization (MALDI)[1] involves the rapid isolation of a desired compound directly on a probe surface from a complex mixture for rapid analysis with mass spectrometry (MS). Hutchens suggested the use of probe surfaces that are designed to extract specific molecules from unfractionated biological fluids and extracts.[2] In one application, agarose beads with attached single-strand DNA were used to capture lactoferrin from preterm-infant urine and were placed directly on the MALDI probe for analysis. To determine the specific components of the protein human basic fibroblast growth factor (bFGF) that interact with mouse mAB 11.1, Chait used agarose beads with the antigen to concentrate partially digested components of the protein.[3] More pertinent to this study is the report by Li and co-workers in which agarose beads containing avidin were used to extract biotinylated peptides and proteins (bradykinin and insulin).[4] The researchers further found that the condition for MALDI preparation caused the degradation of the avidin−biotin complex so that the biotin-labeled compound was deposited on the probe while the agarose beads are physically removed. Avidin immobilized on agarose beads and resins is commercially available. However, it has been noted that agarose beads on the probe surface produce deleterious effects on the MALDI spectrum.[5] A more effective method of producing bioaffinity probes that immobilize affinity sites directly on the probe surface is needed.

Covalent attachment of binding sites on the probe represents a more general approach for producing bioaffinity probes for MALDI MS analysis. Orlando and co-workers developed probe affinity MS to immobilize monoclonal antibody IgG1 directly on the probe so that biotinylated insulin can be captured and analyzed.[6] In the method, antibodies were immobilized onto gold surfaces via their primary amino groups. A newer procedure produced significantly more binding sites; however, the production of these probes requires considerable time and effort.[7]

A simpler and more reliable method for preparing bioactive probes is clearly desirable. Recently, Worrall et al. and Blackledge et al. used proteins adhere to films of microporous polyethylene and polypropylene.[8,9] Vestling et al. and Strupat et al. used polyvinylidene difluoride (PVDF) for similar reasons.[10,11] These procedures take advantage of the natural affinity of the protein for hydrophobic surfaces such as those of plastic films and were used to purify peptides and proteins by adhering the analyte on the film surface and washing away contaminants. MALDI was performed directly on the nonconducting surface to obtain peptide and protein spectra.

If a protein can be immobilized on a surface in a manner similar to that described by Worral, it would serve as a binding site for other substrates. This procedure could then provide a rapid method for the production of bioaffinity probes. Indeed, Liang et al. used a nitrocellulose coating prepared on a MALDI probe to immobilize an antibody, allowing the extraction of the antigen directly from solution. The analysis was completed within 1 h.[12]

In this report, we illustrate an extremely simple and rapid method for immobilizing avidin on polymer films so that biotin/avidin technology can be used to produce a general bioaffinity probe for MALDI. Preparation of the probe and MALDI analysis

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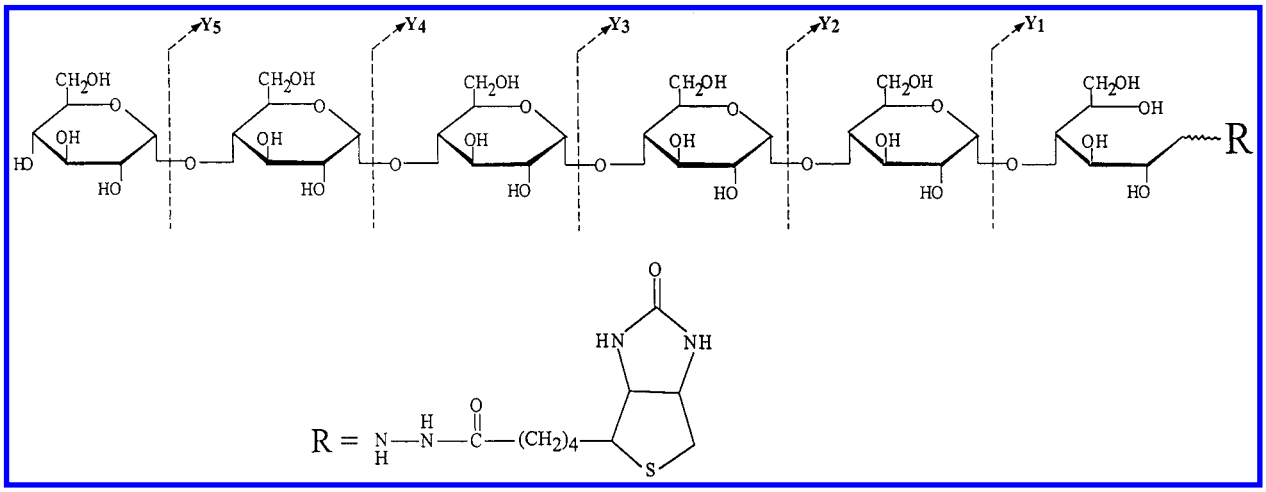
Scheme 1



takes minutes to perform. Oligosaccharides derivatized with biotin are used as sample compounds. Biotin is a relatively small bicyclic compound that contains a tethered carboxylic acid. When it is bound to the protein avidin (from egg white) or strepavidin (from the bacterium Streptomyces avidinii), it produces one of the highest known affinities ($K_a \approx 10^{15}M^{-1}$) between a ligand and a protein. As a consequence, avidin−biotin systems have been widely used as a universal tool for diagnostic purposes.

EXPERIMENTAL SECTION

The mass analysis is performed with Fourier transform mass spectrometry (FTMS). The MALDI-FTMS used for the analysis is a commercial instrument (IonSpec Corp., Irvine, CA) complete with an external MALDI source and a 4.7 T superconducting magnet. A nitrogen laser (standard equipment) operating at 337 nm was used for desorption. A description of the instrument is provided in earlier publications.[13−15]

All oligosaccharides were obtained in the highest purity from commercial suppliers (Sigma, St. Louis, MO and Oxford Glycosystems Ltd., U.K.). Biotin in the hydrazide form and avidin as NeutrAvidin were obtained commercially (Pierce, Rockford, IL). Biotin is available with various lengths between the hydrazide derivatizing group and the biotin group. Two lengths were obtained that differed by an additional seven-membered chain (six carbons and one nitrogen). The shorter length (EZ-Link Biotin Hydrazide) and the longer length (EZ-Link Biotin-LC-Hydrazide) are attached to the oligosaccharide in a similar manner. Polymer films were obtained commercially. The transparency paper is a readily available polyester film (Canon NP transparency film type E) (Canon USA Inc., Lake Success, NY). Microporous polyethylene was obtained from 3M (St. Paul, MN). All other polymer films were obtained as sheets from McMaster-Carr (Los Angeles, CA). Matrixes for MALDI were obtained from Aldrich (Milwaukee, WI) in the highest purity and used without further purification.

A procedure developed by Takao and co-workers for attaching 4-aminobenzoic acid 2-(diethylamino)ethyl ester to neutral oligosaccharides was adapted for the synthesis of the biotinylated oligosaccharides.[16] This involves forming the Shiff base and reducing the intermediate with sodium cyanoborohydride to produce the corresponding amine. Biotinylation of oligosaccharides was performed by adding 2.6 mg (10 μmol) of biotin hydrazide in 80 μL of methanol to sodium cyanoborohydride (3.5 μg) in a 1.5-mL polypropylene microcentrifuge test tube. Glacial acetic acid (4 μL) was then added to the solution to form the reagent mixture. The reagent mixture was added to the solution of an oligosaccharide (200−400 nmol) in 40 μL of H_2O. Methanol was further added to give a total volume of 200 mL. The reaction tube was heated at 80 °C for 2 h. The reaction mixture was then distilled with methanol to remove the boron. This procedure must be performed diligently as any amounts of boron will form complexes with the oligosaccharide to produce spurious peaks and poor sensitivities. The resulting biotinylated oligosaccharide was separated from excess reagent by HPLC on a primary-amine-bonded silica column, using a 70:30 acetonitrile/water mixture as a mobile phase. The eluent was collected in fractions.

RESULTS

Biotinylated Oligosaccharides. Shown in Scheme 1 is the structure for maltohexaose derivatized with biotin hydrazide. The biotin shown contains the short tether form. Under reaction conditions consisting of a 10:1 biotin/oligosaccharide ratio, the major product is the reduced biotinylated derivative. The only other component observed by MALDI and HPLC is unreacted oligosaccharide (<5%). The derivatization reaction is easily followed by MALDI. The MALDI-FTMS of biotinylated oligosaccharide generally produced stronger signals than that of the underivatized compound when the compounds are analyzed under standard MALDI conditions with a stainless-steel probe. A similar observation is also made with other oligosaccharides derivatized at the reducing end.[17] The MALDI-FTMS spectrum of maltohexaose, a hexasaccharide linked via α(1−4), purified by HPLC,

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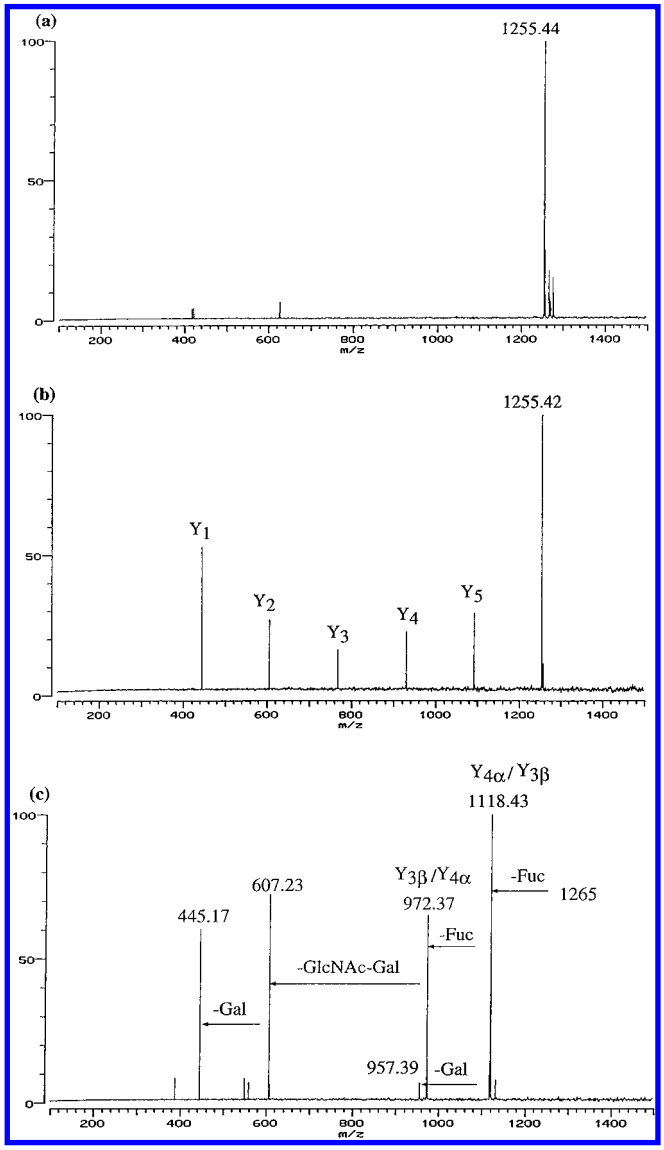
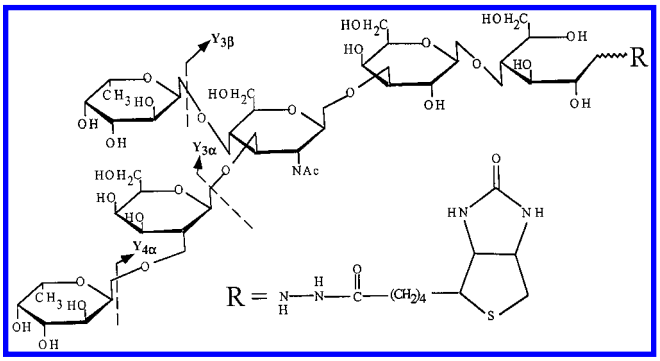


Figure 1. (a) MALDI-FTMS spectrum of biotinylated maltohexaose produced by the procedure outlined in the Results Section. See text for details. (b) MALDI-CID-FTMS spectrum of biotinylated maltohexaose. (c) MALDI-CID-FTMS spectrum of biotinylated LNDFH-I.

shows the quasimolecular ion primarily, i.e., the sodium adduct of the parent (Figure 1a). To confirm the structure of the product, CID was performed by first isolating the quasimolecular ion. The CID spectrum of the biotinylated maltohexaose shows fragmentation occurring at every glycosidic bond to produce a series of y-type ions, i.e., ions with the reducing end attached (Figure 1b, see also Scheme 1). The presence of only y-type cleavages provides an ideal situation for sequencing and suggests that the Na^+ is sequestered primarily at the reducing end. Apparently, labeling the oligosaccharide with biotin renders the reducing end a strong chelator of Na^+. Thus, derivatization with avidin has an added feature of providing sequence and allowing differentiation between the reducing and nonreducing ends. For comparison, maltohexaose was reduced using sodium borohydride reduction to produce the corresponding alditol without the biotin label. The CID of this compound produced ions due to both b- and y-type cleavages, suggesting a less specific coordination of the Na^+ to both reducing and nonreducing ends (spectrum not shown).

Scheme 2



Biotinylated branched oligosaccharides also have characteristic fragmentation that yields the branch points in the molecule. For example, LNDFH-I (Scheme 2), also a hexasaccharide, fragments to produce the loss of fucose (m/z 1118.44, $Y3_\beta$ or $Y4_\alpha$) followed by a loss of another fucose (m/z 972.37, $Y3_\beta$ or $Y4_\alpha$)) or a loss of galactose (m/z 957.39, $Y3_\alpha$) (Figure 1c). The loss of both fucose and galactose yields the branching at the *N*-acetylglucose residue. The loss of *N*-acetylglucose (m/z 607) and the loss of the other galactose (m/z 445.17) provide the complete sequence.

Immobilization of NeutrAvidin on Membrane Surface. Unlike other methods of surface immobilization of avidin which require the formation of covalent bonds, the avidin is attached in this procedure by taking advantage of the natural ability of proteins to adhere to hydrophobic surfaces. Preparation of the film is simplified considerably and mainly involves drying the protein solution on the film.

The entire process takes several minutes. The film is first cut into size and taped with cellophane tape to the stainless-steel probe. The surface is wiped with ethanol-soaked cotton tips and allowed to air dry. Once the film is dry, a 1-μL solution (2 mg/mL in H_2O) of NeutrAvidin, a cheaper and deglycosylated form of avidin, is placed directly on the film. The solution is allowed to air dry either under ambient conditions or with forced air. Drying the avidin fixes the protein on the film surface. We find that the avidin-treated film retains its activity even after several washings with water. As many as 15 washings have been performed with 40 μL per wash with no loss of activity observed. A major concern was whether avidin retained its activity after being dried on the surface. The studies described below will show that avidin dried on a surface remains active toward biotin.

MALDI-FTMS from avidin-immobilized film. Sample preparation involves addition of 1 μL of analyte solution (≈0.8 mg/mL) in 70:30 acetonitrile/H_2O to the treated film surface. For the experiments detailed in this section, a commonly available transparency film known as Cannon NP transparency Film type E was used. The discovery of this film as a MALDI probe surface was fortuitous. We had intended to use it as an experimental blank but found it to have excellent properties for binding avidin. Approximately 5 min was allowed for the biotin−avidin binding to take place. This appeared to be the optimal period for binding. With a shorter time the signal is attenuated while with a longer time no further signal enhancement is observed. The transparency was then washed with Nanopure water (10 times at 40 μL per washing) to remove the nonbinding components. For neutral oligosaccharides, a 1 μL aliquot of 0.01 M NaCl solution was added

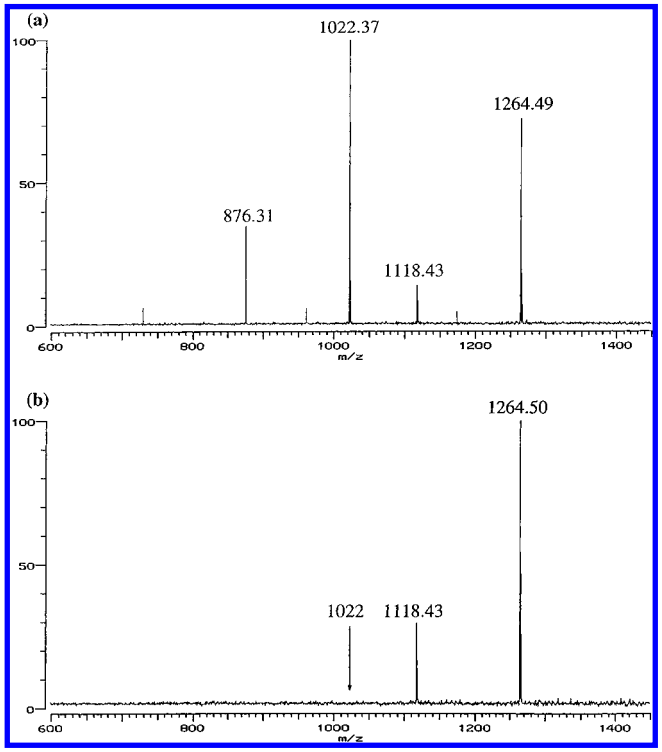


Figure 2. (a) MALDI-FTMS spectrum of a mixture of biotinylated (*m*/*z* 1264.49) and native (*m*/*z* 1022.37)) LNDFH-I from a control probe surface. (b) Competition experiment involving the mixture in (a) on an avidin-treated surface. Only the biotinylated compound is observed in the mass spectrum. The native compound (arrow) is absent.

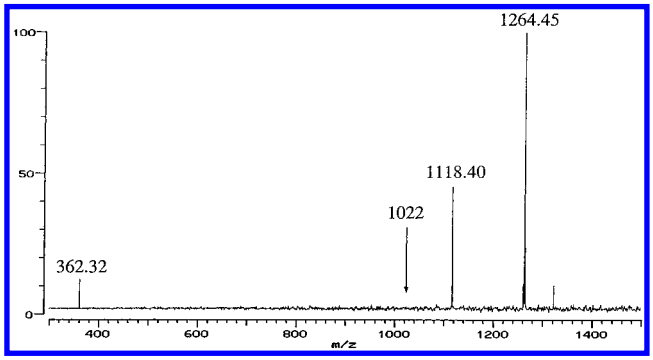


Figure 3. Competition experiment involving equimolar amounts of biotinylated and native LNDFH-II. Only the biotinylated material is observed (*m*/*z* 1264.45) in the MALDI-FTMS spectrum. The native compound (arrow) is absent.

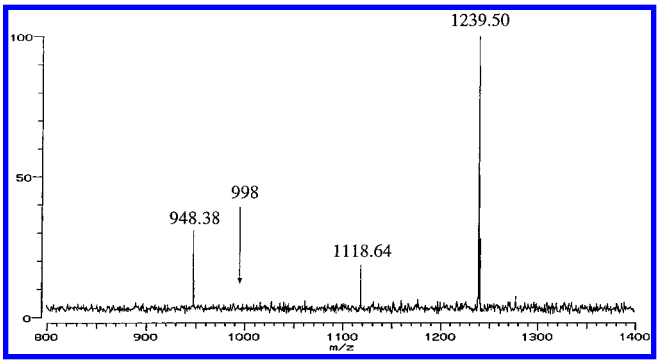


Figure 4. Competition experiment involving equimolar concentrations of biotinylated and native LST-a, a hexasaccharide containing sialic acid. Only the biotinylated material is observed (*m*/*z* 1239.50) in the mass spectrum. The other peaks correspond to fragments of the quasimolecular ion. The native compound (arrow) is absent.

on the probe tip as a cation dopant to produce primarily sodiated species. This was followed by 1 μL of the matrix solutions consisting of 50 mg/mL of 2,5-dihydroxybenzoic acid (DHB). The mixture was then dried with warm forced air. For MS analysis, the probe was inserted directly into the MALDI source of an external-source FTMS instrument.

Competition experiments were performed with biotinylated and free oligosaccharide on avidin-treated (bioaffinity) and untreated (control) surfaces. The control surface was prepared by treating the film as described above but with only an acetonitrile/H_2O solution (i.e., no avidin). An aqueous solution containing 50:50 biotinylated and native LNDFH-I was applied to the treated and the control surfaces. Figure 2a shows the MALDI-FTMS spectrum of the mixture applied to the control surface without the washing procedure. Both biotinylated (*m*/*z* 1264.49) and native LNDFH-I (*m*/*z* 1022.37) are present in nearly equal abundances. The other less intense signals (*m*/*z* 1118.43 and 876.31) are due to fragmentation via loss of fucose from the quasimolecular ions. Washing the surface with water before the solution is dried removes both compounds as no signal is observed in the MALDI spectrum (spectrum not shown).

The sample on the avidin-treated surface was further washed with Nanopure water as before to remove the uncomplexed component, dried, and treated with the matrix solution. Figure 2b shows the MALDI-FTMS spectrum of the avidin-treated surface. The native oligosaccharide (arrow) is not observed and is presumably washed away, while the biotinylated oligosaccharide is retained as the major peak in the spectrum. The S/N ratio is less for the avidin-treated surface as the amount of avidin that can be bound to the surface similarly limits the amount of biotinylated oligosaccharide.

Several oligosaccharides of varying structures and sizes were also examined. LNDFH-II, an isomer of LNDFH-I, was analyzed under identical conditions. Again, only the biotinylated material was retained (*m*/*z* 1264.45) after the washing. The native compound is not observed in the mass spectrum (*m*/*z* 1022, Figure 3). Note the strong abundance of *m*/*z* 1118.40, which corresponds to the loss of fucose. Fucose loss is a common fragmentation of fucosylated oligosaccharides. However, the fragmentation seems slightly more intense from the bioaffinity probes as compared with the standard stainless-steel probes. It generally takes a greater photon density to desorb the material, thereby causing slightly more fragmentation.

A smaller oligosaccharide, the trisaccharide 2-fucosyllactose, was also examined (spectrum not shown). Again, only the biotinylated compound is observed (*m*/*z* 753.27) with MALDI, while no trace of the native compound (*m*/*z* 510) is observed.

The probe also works well for acidic oligosaccharides. A sialylated hexasaccharide, LST-a, and the sialylated trisaccharide, 6′-sialyllactose, were examined with the bioaffinity probe and MALDI. The acidic hexasaccharide, analyzed in the anion mode, exhibits the same specificity as the neutral oligosaccharides in the cation mode (Figure 4). Only the biotinylated compound (*m*/*z* 1239.50) and its fragments are observed, while no native species is observed (*m*/*z* 998). The trisaccharide also exhibits the same behavior (spectrum not shown).

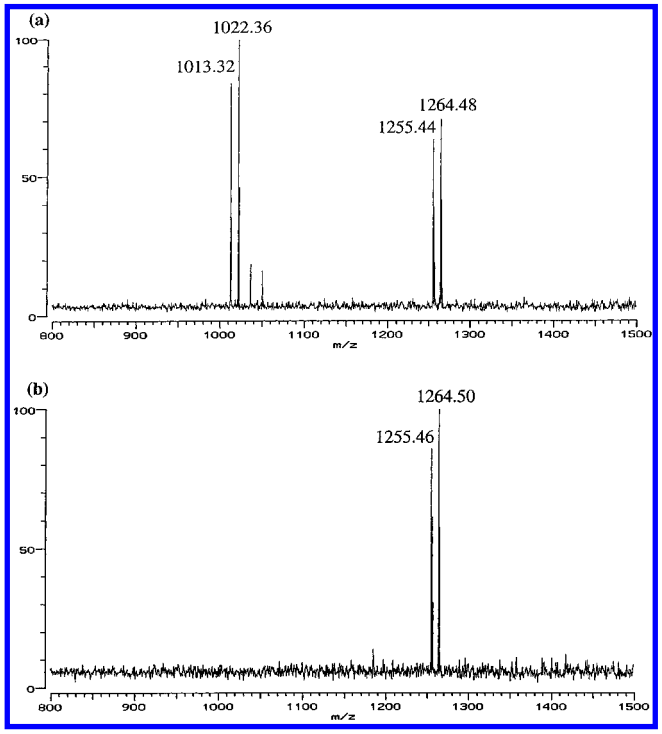


Figure 5. (a) MALDI-FTMS spectrum of a mixture of biotinylated maltohexaose (*m*/*z* 1255.44) and LNDFH-I (*m*/*z* 1264.48) with their native analogues (*m*/*z* 1013.32 and 1022.36, respectively). (b) MALDI-FTMS of the same mixture on a bioaffinity MALDI probe. Only the biotinylated species are observed.

Ideally, the probe should not differentiate between oligosaccharides of different structures. Experiments with a mixture composed of a linear and a branched oligosaccharide (both biotinylated and native) were performed to examine whether the probe exhibits preference for either linear or branched structure. Maltohexaose and LNDFH-I are both hexasaccharides, with LNDFH-I being branched (Schemes 1 and 2). A solution mixture containing biotinylated and native maltohexaose and biotinylated and native LNDFH-I was made and applied to the control surface and dried. The spectrum in Figure 5a shows the quasimolecular ion of biotinylated (MNa^+, m/z 1255.44) and native (m/z 1013.32) maltohexaose along with biotinylated (m/z 1264.48) and native (m/z 1022.36) LNDFH-I, all in nearly equal abundances. An identical mixture was placed on the avidin-immobilized probe and washed as before with water. The resulting spectrum after applying matrix and NaCl shows only the biotinylated oligosaccharides in essentially the same relative abundances (Figure 5b). Biotin/avidin interactions are apparently not affected by branching on the oligosaccharides.

To optimize the analytical conditions, a few commonly used MALDI matrixes were examined to determine the best conditions for performing MALDI from the bioaffinity probe. In addition to DHB, super DHB (50 mg/mL of DHB with 10% 2-hydroxy-5-methoxybenzoic acid), α-cyano-4-hydroxycinnamic acid, and sinapinic acid were examined. The relative scale (normalized to the DHB) of biotinylated LNDFH-I is provided in Table 1. We find that DHB performed the best by providing the strongest quasimolecular ion and producing the least fragmentation. Super DHB performed slightly worse, while α-cyano-4-hydroxycinnamic acid produced primarily fragment ions and sinapinic acid produced essentially no signal.

Table 1. Relative Intensity of Biotinylated LNDFH-I on the Bioaffinity MALDI Probes with Different Matrixes

matrix	relative intensity of quasimolecular ion
2,5-dihydroxybenzoic Acid (DHB)	1.0
super DHB	0.62
α-cyano-4-hydroxycinnamic acid	fragments only
sinapinic acid	no signal

We should also point out that the biotinylated material is retained on the untreated surface (i.e., no avidin) if the sample is allowed to dry. Washing the dried surface, the subsequent analysis with MALDI shows strong intensity of the biotinylated oligosaccharides. A similar experiment with a nonbiotinylated oligosaccharide also shows underivatized oligosaccharides in the MALDI spectra. Evidently, the polymer surface binds both biotin and native oligosaccharides upon drying; however, if the sample is not allowed to dry but is washed, even after the exposure period, no retention of either oligosaccharide is observed (spectra not shown). The binding of biotin to polyester may also be used to clean biotinylated oligosaccharides in the future.

Determination of Biotin/Avidin Ratio on the Probe Surface. Concentration studies were performed with biotinylated LNDFH-I to determine the optimal amount of avidin that is immobilized on a 1 mm^2 probe surface. Concentrations of the avidin including 0.5, 1.0, 2.0, and 4.0 mg/mL were used to prepare the bioaffinity probe. Introduction of the biotinylated substrate and the subsequent MALDI produces spectra (not shown) with relative intensities for the quasimolecular ion increasing in the order 1.0, 4.2, 6.3, and 4.5. The optimal concentration is 2.0 mg/mL, which corresponds to 34 picomoles applied to the probe tip. The intensity of the quasimolecular ion obtained from this concentration was quantified by matching the signal with known amounts of biotinylated oligosaccharides placed on a stainless-steel probe. The intensity of the quasimolecular ion corresponds approximately to 40 picomoles of biotinylated material. This translates to a 1.2:1 ratio of biotin to avidin. A similar ratio was reported for agarose-immobilized avidin.[4] Avidin can bind theoretically with four biotin molecules. However, the film surface renders at least one site completely inaccessible. Of the remaining sites, two others are near the surface, which may inhibit binding of biotin attached to large groups such as oligosaccharides.

MALDI of Biotin with Longer Spacers. It has been suggested that longer spacers increase the biotin/avidin ratio with immobilized avidin.[4] We performed similar studies using the longer spacers of Biotin-LC−Hydrazide. Figure 6a shows the MALDI of a mixture of LNDFH-I with the short tether (m/z 1264.48) and with the long tether (m/z 1377.55) on a standard-stainless-steel probe. When the samples are placed on the bioaffinity probe and treated as described, we find that the intensity of the long-tether compound increases by over a factor of 3 relative to that of the short-tether compound (Figure 6b). Indeed, increasing the spacer length between the hydrazide group and the biotin increases the number of substrates retained on the avidin. However, a factor of 3 is often not a significant improvement in MALDI, so that the less expensive short tether is judged sufficient for the analysis.

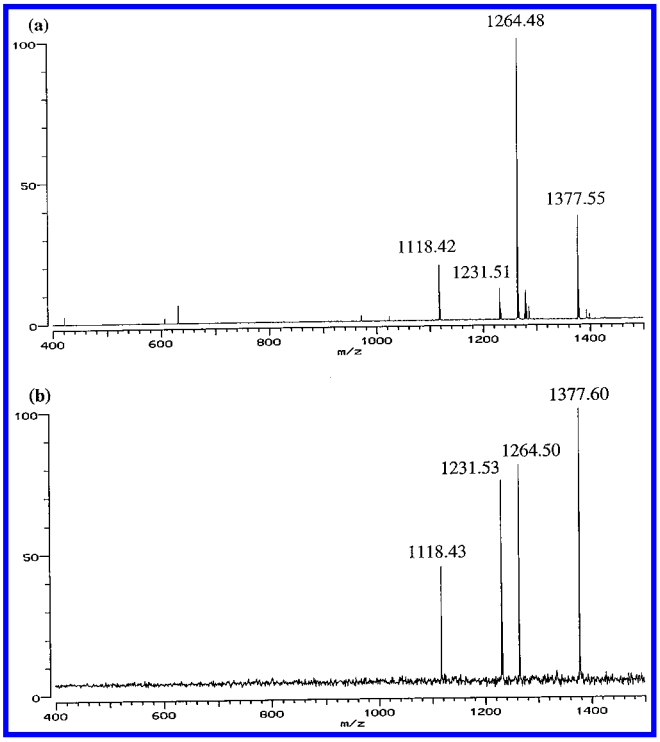


Figure 6. (a) MALDI-FTMS of biotinylated LNDFH-I with short (*m*/*z* 1264.48) and long (*m*/*z* 1377.55) tethers. (b) MALDI-FTMS of same mixture on bioaffinity MALDI probe. The signal for the long tether (*m*/*z* 1377.60) is enhanced relative to that of the short tether (*m*/*z* 1264.50). The peaks with *m*/*z* 1231 and 1118 are due to losses of fucoses from the quasimolecular ions.

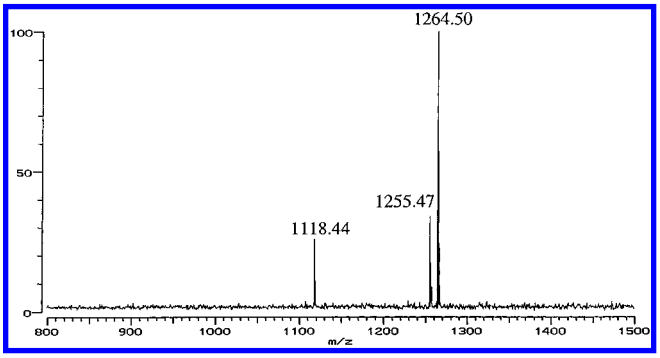


Figure 7. MALDI-FTMS spectrum of the bioaffinity probe saturated with biotinylated LNDFH-I followed by biotinylated maltohexaose. Approximately 35% of the LNDFH-I is replaced on the surface by maltohexaose.

Table 2. Relative Intensity (Normalized to the Transparency-Film Spectrum) of LNDFH-I with Bioaffinity MALDI Probes Produced from Several Polymer Films

membrane material	relative intensity of quasimolecular ion
transparency film	1.0
microporous polyethylene	0.94
low-density polyethylene	0.59
high-density polyethylene	no signal
polyester	0.25
polyvinylidene fluoride (PVDF)	no signal

Biotin−Avidin Equilibrium on the Film Surface. To determine the nature of the equilibrium of the biotin−avidin interaction, experiments were performed by saturating the avidin-treated surface with one biotinylated oligosaccharide and exposing the surface to a second biotinylated oligosaccharide. In this experiment, biotinylated LNDFH-I was placed on the treated probe for 5 min and washed with water. Another solution of biotinylated maltohexaose was placed on the probe for an additional 5 min and washed again with water. The NaCl solution was added as dopant, followed by DHB as matrix, and the surface was dried. The MALDI spectrum shows the intensity of maltohexaose (*m*/*z* 1255.47) to be about 35% that of LNDFH-I (*m*/*z* 1264.50) (Figure 7). The reverse experiment yields similar results (spectrum not shown). The binding of avidin to biotin is indeed strong but remains in equilibrium so that free biotin molecules can displace those already coordinated. Furthermore, it shows that the avidin remains active toward biotin during the preparation process as assumed. The results, however, serve as a caveat for this method; that is, excess biotin may attenuate the intensity of the desired biotinylated species by displacing them on the surface.

Comparison of Various Polymer Films. Several polymer surfaces were used to find the best surface for binding avidin. In addition to the transparency film, we examined films of polyester, microporous polyethylene, low- and high-density polyethylene, polyester, and polyvinylidene fluoride (PVDF)—a film commonly used to immobilize proteins in biochemical applications. The brand of transparency paper is mainly composed of polyester, while microporous polyethylene was used by Woods et al. to immobilize proteins for MALDI-MS.[9]

Table 2 lists the relative abundance of biotinylated LNDFH-I with the various films under identical concentrations and conditions. The relative abundance of LNDFH-I illustrates the efficacy of the surface as a bioaffinity probe for neutral oligosaccharides. The relative intensity is undoubtedly related to the ability of the surface to immobilize the protein avidin. Both the transparency film and the microporous polyethylene performed the best for the neutral oligosaccharides. The two surfaces produce nearly the same intensity of biotinylated LNDFH-I under MALDI conditions. Low-density polyethylene and pure polyester films are readily available and are considerably cheaper but will work to some degree. Interestingly, pure polyester does not perform as well as the transparency film.

CONCLUSION

Although the procedures for preparing the MALDI probe and the MS analysis are simple, several events must take place in the preparation and the MALDI analysis for it to be successful. First, the avidin must adhere to the surface and remain active after drying so that biotin attaches to the surface. Second, the oligosaccharide must be able to compete for charge (usually in the form of Na^+) with the protein. Third, the complex must dissociate before detection. The final point is often noted as a disadvantage of MALDI. Noncovalently bound complexes are generally not observed in MALDI with few exceptions. None have been

(18) Ngoka, L.; Lebrilla, C. B. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 210−215.

observed with MALDI-FTMS. This may be due to the relatively high energy associated with MALDI and the long time scale of the FTMS technique that allows metastable ions to dissociate before detection.[18,19] The time scale between ion formation and ion detection is particularly long in FTMS where it may last several seconds. The dissociation is necessary to obtain the oligosaccharide component. We point out, however, that Li and co-workers have also suggested that the complex dissociates under the chemical conditions of MALDI.[4] By applying the matrix, they were able to remove the agarose beads because the complex had dissociated and moved to the MALDI surface. Both chemical and thermal dissociation of the complex probably occurs in the analysis.

(19) Ngoka, L. C.; Gal, J. F.; Lebrilla, C. B. *Anal. Chem*. **1994**, *66*, 692−698.

Finally, we have shown applications to oligosaccharides, although this method will work with any biotinylated compound. Other biopolymers, including peptides and proteins, can be analyzed in the same manner. The advantage of this process is that the probe can be prepared in a few minutes and with high reliability because it does not require the formation of covalent bonds.

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