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Enhanced Affinity Capture MALDI-TOF MS: Orientation of an Immunoglobulin G Using Recombinant Protein G

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Although immobilization of antigen-specific immunoglobulins onto matrix-assisted laser desorption/ionization (MALDI) targets allows the specific detection and enrichment of an antigen from complex biological fluids, the process of antibody immobilization is not optimal. The principal reason is that the antibody can bind to the template in various orientations, many of which block antigen recognition. An affinity capture MALDI mass spectrometry methodology was developed by covalently immobilizing an F_c receptor (recombinant protein G) onto MALDI gold targets for the purpose of orientating an immunoglobulin G, with the F_{ab} domains pointing away from the target surface. The pregnancy and cancer marker, human chorionic gonadotropin β core fragment (hCGβcf), was our chosen test substance. To optimize the methodology, different surface densities of protein G and immunoglobulin were achieved by employing varying concentrations for immobilization. Captured amounts of hCGβcf were compared using an external standard (cytochrome *c*). Orientation of immunoglobulin resulted in an ~3-fold increase in MALDI signal compared to using randomly immobilized antibody. Higher antibody concentrations resulted in diminished MALDI signals, which were explained by steric hindrance. Purification and enrichment of hCGβcf was achieved from a test solution containing contaminant peptides and proteins using oriented immunoglobulins on-target.

Immunoaffinity methods and mass spectrometry are established techniques found in many bioanalytical laboratories. Their combination in the form of affinity capture mass spectrometry has the potential to detect, isolate, and identify a vast range of target analytes in a very simple manner. For protein analysis, various types of immunoaffinity separations are combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS), with antibody−antigen interactions being the most often employed. Affinity capture (AC) MALDI MS has also been applied to study peptide−metal ion affinity,[1,2] lectin and carbohydrate affinity of microorganisms,[3] and genetic polymorphisms.[4,5] In addition, peptide and protein digestion was performed on enzymatically active MALDI probes, yielding an increased sensitivity and less enzymatic autolysis fragments.[6,7] Under the MALDI conditions, the noncovalent biospecific interactions between binding molecule and analyte are disrupted and the captured biomolecule can then be analyzed in the mass spectrometer.[8] Frequently used "indirect" methods imply immobilization of a binding molecule on a solid matrix, i.e., on a solid support for immunoaffinity chromatography or on magnetic beads,[2,9−11] which are then placed on a conventional MALDI target for mass analysis. A practical development is the use of a "direct" methodology where a binding molecule is immobilized on a MALDI target for affinity capture of a protein.[12−15] The direct AC MALDI MS approach seems to be superior to the indirect method because the latter has been shown to degrade the MALDI performance.[2]

Different strategies have been used to immobilize antibodies on a MALDI target. The affinity of proteins to hydrophobic polymer films, i.e., nitrocellulose, is exploited to produce AC MALDI probes with immobilized antibody[15] or streptavidin.[10]

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Major drawbacks of this approach are the random immobilization of antibody and the predisposition to nonspecific binding of other proteins to the nitrocellulose surface when complex solutions are analyzed.[15] Alternatively, methods for covalent activation of MALDI targets were investigated by many researchers. Activation can be achieved by using the cleavable homobifunctional linker dithiobis(succinimidyl propionate) (DTSP) on gold-coated MALDI surfaces.[6,13,16] Anchoring of the DTSP linker molecule onto MALDI targets is based on the dissociative chemisorption of its disulfide group on gold surfaces, the basic chemistry of which was first reported by Katz for the modification of gold electrodes.[17] The *N*-hydroxysuccinimide active ester functionalities of the monolayer are used to immobilize biomolecules via their primary amino groups. Self-assembled monolayers[18] (SAM) organized from DTSP have also been successfully employed in biosensor applications for covalent immobilization of biomolecules.[19] For AC MALDI purposes, it was soon realized that the loading capacity of the antibody surfaces on DTSP SAM was limited, it being reasoned that the relatively short length of the linker sterically hinders antibody immobilization and accessibility of the antigen to the binding sites.[14] To overcome this problem, Brockman and Orlando proposed a new antibody immobilization scheme that is not restricted by monolayer formation; however, the assembly of their affinity probes takes several days.[14]

The number and accessibility of available antibody binding sites on the surface of AC MALDI MS targets is now recognized as a crucial factor for improving detection capabilities. A strategy of improving the loading capacity of an AC MALDI surface can be by (I) ensuring the correct orientation of the antibody and (II) optimizing antibody surface density. (I) Orientation of antibodies, i.e., γ-immunoglobulins, can be accomplished by binding to F_c receptors on solid supports, such as protein A, protein G, or recombinant protein A/G, as reported for immunoassays, immunosensors, and immunoaffinity chromatography.[20−22] Use of protein G for antibody immobilization is particularly attractive because a wide range of γ-immunoglobulins can be bound strongly, with affinity constants ranging from 10^9 to 10^{10} M^{-1}.[23] In addition, recombinant variants of protein G are commercially available that are particularly suitable for covalent immobilization on solid support. (II) An optimized antibody surface density is desirable because the distance between bound antibodies and the solid support influences the antigen-binding capacity due to steric hindrance.[20−22,24] However, to the best of our knowledge, both these factors have not been described in the peer-reviewed literature with regard to AC MALDI MS. There is a need to investigate these aspects further in order to obtain larger signals for on-target affinity-purified peptides and proteins. In addition, future availability of immobilized F_c receptors (such as protein G) on MALDI targets could be of benefit to those who do not want to perform surface chemistry but have a need to link antibodies to MALDI targets rapidly.

Here we report the development of an optimized affinity capture MALDI MS system using as our chosen test substance the core fragment of the β subunit of human chorionic gonadotropin (hCGβcf), a major metabolite of the glycoprotein human chorionic gonadotropin, its current detection of this cancer and pregnancy marker being by immunoassay. Recently there has been an interest in identification of this analyte by mass spectrometry. To capture hCGβcf, DTSP self-assembled monolayer technology was used to covalently immobilize recombinant protein G for antibody orientation onto a gold-coated surface of a MALDI target. The effects of antibody orientation and antibody density on the differences in MALDI signal intensity after incubation with the antigen, followed by extensive washing, were measured. This was achieved by adding an external standard (cytochrome *c*) on the target together with the MALDI matrix to compare relative signal areas.[25,26] To determine the effectiveness of antibody orientation, data were compared to that obtained using the same monoclonal antibody randomly immobilized to the SAM on the gold surface.

EXPERIMENTAL SECTION

Materials. Dithiobis(succinimidyl propionate) was purchased from Sigma (Poole, U.K.). Anhydrous dimethyl sulfoxide (DMSO) and anhydrous ethanol, both over molecular sieves, were obtained from Fluka (Buchs, Switzerland). Trifluoroacetic acid (TFA) and acetonitrile were of analytical grade and from BDH (Poole, U.K.) and Rathburn (Walkerburn, Scotland), respectively. Sinapinic acid was purchased from Aldrich (Gillingham, U.K.) and was recrystallized from hot ethanol prior to use. Water was purified on an Elgastat UHQ system (High Wycombe, U.K.). Monoclonal antibody directed against human chorionic gonadotropin (hCG MAb 147-PA) was obtained from Akzo Nobel). The affinity constants of this antibody for hCG, β subunit of hCG, and hCGβcf are quoted as being 1.8×10^9, 2.1×10^9, and 5.6×10^9 M^{-1}, respectively. Recombinantly engineered protein G was obtained from Calbiochem (Nottingham, U.K.). For cross-linking purposes, an extra 10 lysine residues were added to the highly charged C-repeats of the protein (distinct from the B-repeats for antibody binding). The hCGβcf preparation was a kind gift from Professor Laurence A. Cole (University of New Mexico). Oxidized bovine insulin chain B, horse heart cytochrome *c* (CytC), bovine α-lactalbumin, bovine carbonic anhydrase, and bovine serum albumin were purchased from Sigma. Recombinant human growth hormone was obtained from Serono Pharmaceuticals Ltd. (Feltham, U.K.). MALDI autosampler strips consisting of 48 targets, these being stainless steel disks with an anodized surface diameter of 2.2 mm, were supplied by Thermo Bioanalysis Ltd. (Hemel Hempstead, U.K.).

Preparation of Immunoaffinity Disks. MALDI autosampler strips were located in a customized jig, and the stainless steel disks were punched out. A gold layer of ~30-nm thickness was sputtered onto the scratched sides of the disks using a SEM Coating Unit E5100 (Polaron Equipment Ltd., Watford, U.K.). The

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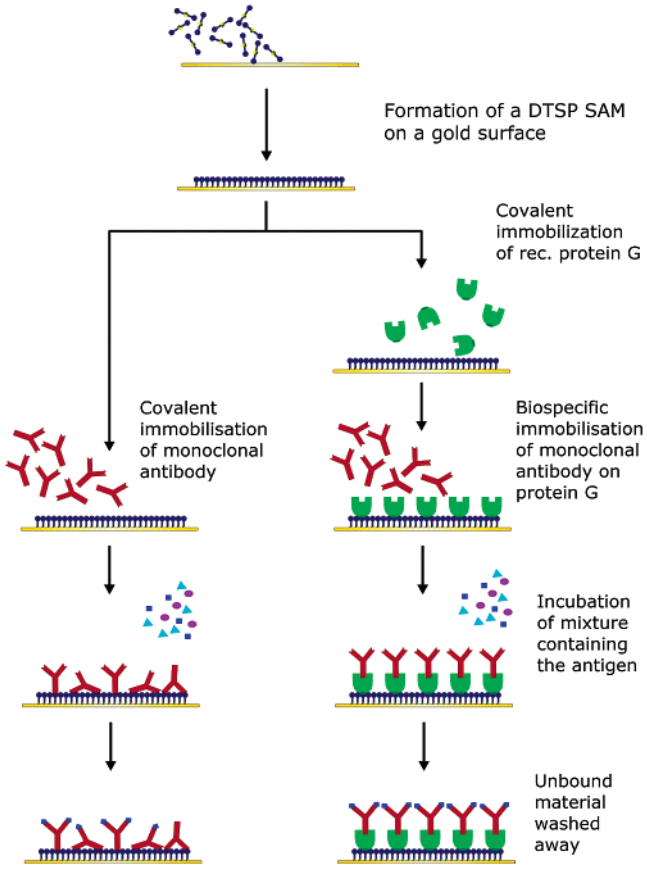

Figure 1. Schematic representation of the preparation of the immunoaffinity disks (left, random immobilization of immunoglobulin; right, oriented immobilization of immunoglobulin using recombinant (rec.) protein G).

preparation of the immunoaffinity disks is schematically presented in Figure 1**.** A self-assembled monolayer was prepared by dissociative chemisorption of the dialkyl disulfide DTSP onto the gold surface,[17] the likely mechanism of anchoring being by disulfide bond cleavage and subsequent formation of gold thiolate species (ref 18 and references therein). To this purpose, each sputtered disk was transferred into a polypropylene tube (39 mm height × 10 mm diameter), 200 μL of anhydrous DMSO containing 10 mM of DTSP was added, and incubation was performed at 20 °C for 1.5 h. The disks were then washed twice with 200 μL of anhydrous ethanol.

For producing immunoaffinity disks that have only antibody bound to the surface, i.e., randomly immobilized antibody, the SAM was reacted with 100 μL of hCG MAb in phosphate-buffered saline (PBS), at concentrations ranging from 100 ng/mL to 100 μg/mL, at 20 °C for 16 h. For producing immunoaffinity disks that accommodate oriented antibody on a protein G monolayer, i.e., oriented antibody, the SAM was reacted with 100 μL of recombinant protein G in PBS (5.0, 1.0, or 0.5 μg/mL) at 20 °C for 16 h. Following aspiration, 100 mL of hCG MAb in PBS in concentrations ranging from 100 ng/mL to 100 μg/mL was added and incubated at 20 °C for 3 h. After random or oriented immobilization of hCG MAb, the disks were washed with 200 mL of 0.5% (w/v) Triton X-100 in PBS and twice with 200 μL of PBS to ensure that all nonspecifically bound proteins were removed. The immunoaffinity disks were not allowed to dry in the tubes in order to prevent the possibility of denaturation of the immobilized protein G or the antibody. Following preparation, the disks were left in the tubes for subsequent immunoaffinity capture and washing steps, which were performed on the same day.

Development of Detergent Washing Conditions. The effect of washing with different concentrations of the nonionic detergent Triton X-100 was investigated on random immobilized antibody disks (prepared using 5 μg/mL hCG MAb; as described in the previous section). Gold disks without linker, but having been incubated with the same concentration of antibody, and untreated gold disks underwent the same washing process for control purposes. The disks were incubated with the antigen hCGβcf (10 pmol/μL; 20 μL) in PBS by shaking for 1.5 h at 20 °C.

Immunoaffinity Capture of Antigen. The immunoaffinity disks were either incubated in 20 μL of solution of hCGβcf in PBS by shaking for 1.5 h at 20 °C (the antigen concentration ranging from 2 pmol/μL to 100 fmol/μL) or in 20 μL of a test solution containing several peptides and proteins. The test solution contained oxidized chain B of insulin (200 fmol/μL), hCGβcf (1000 fmol/μL), bovine α-lactalbumin (400 fmol/μL), recombinant human growth hormone (800 fmol/mL), bovine carbonic anhydrase (800 fmol/μL), and bovine serum albumin (2000 fmol/μL) in PBS. The disks were then washed once with 200 μL of 0.5% (w/v) Triton X-100 in PBS, twice with 200 mL of PBS, and finally with 200 μL of water to remove buffer salts, which are likely to interfere with the MALDI process,[27] and then left at room temperature until dry.

MALDI TOF Mass Spectrometry. The immunoaffinity disks were affixed to the mass spectrometer autosampler strip using 1-mm^2 squares of double-sided tape. A solution of 20 mg/mL sinapinic acid in 40:60 acetonitrile/0.05% (v/v) TFA−water was prepared containing cytochrome *c* (125 fmol/μL) for standardizing MALDI signal areas. Cytochrome *c* was selected because of its molecular weight of ~12 200 (same range as hCGβcf) and because it gives rise to intense MALDI signals even at low concentrations; therefore ion suppression was not expected. An 0.8-μL aliquot of this mixture was added onto each disk by pumping the micropipet three times to promote mixing. In this way, the molar ratios between cytochrome *c* and sinapinic acid were kept constant. The sample surfaces were crystallized on air before analysis using a Lasermat 2000 MALDI-TOF mass spectrometer (Thermo Bioanalysis Ltd.). Pulsed light from a nitrogen laser (λ_{max} = 337 nm) was used to desorb ions from the samples, which were then accelerated by a 20-keV electric field down a linear 0.5-m drift tube and detected by a microchannel plate detector. The detector signal was digitized at a sampling rate of 500 MHz and transferred to a PC for data analysis. To account for heterogeneity within a MALDI sample,[28] spectra were generated across the target from 20 adjacent laser aims, firing 15 shots per aim equating to 300 shots per spectrum.

Data Processing. Saved data files were exported from the Lasermat Data Review software as ASCII files and imported into GRAMS Spectral Notebase. After smoothing and baseline subtraction, the signals for the singly charged molecular species of hCGβcf and CytC were integrated. To compare relative signal intensities, the ratio of the signal areas was calculated.

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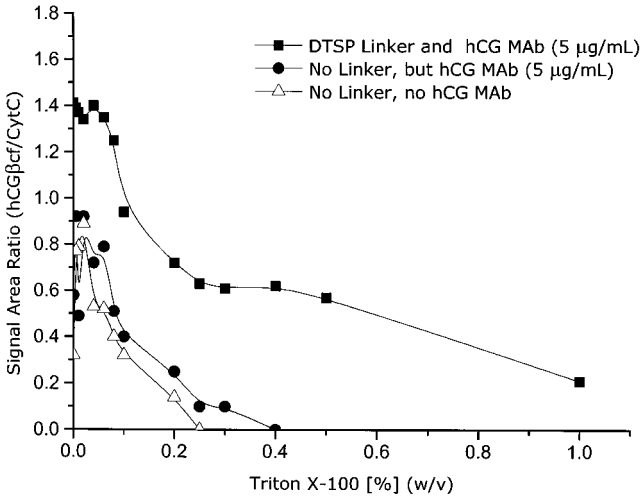

Figure 2. Effect of washing with different concentrations of the surfactant Triton X-100 following incubation with hCGβcf (10 pmol/μL) on the signal area ratio. These experiments were carried out in duplicates. (untreated gold targets, ■; gold targets without linker but with antibody, ●; and gold targets with covalently immobilized antibody, △).

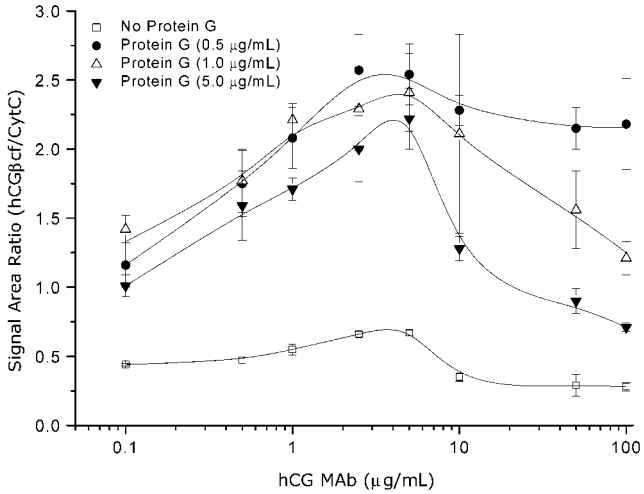

Figure 3. Immobilization of different concentrations hCG MAb on either DTSP activated gold disks (□, random covalent immobilization of MAb) or disks where recombinant protein G was covalently attached to a DTSP self-assembled monolayer on gold for biospecific immobilization of hCG MAb. Protein G concentration ranged from 0.5 (●), 1.0 (△), to 5.0 μg/mL (▼) (oriented immobilization). Immunoaffinity disks were incubated with 20 μL of 1 pmol/μL hCGβcf ($n = 3$) in PBS by shaking for 1.5 h at 20 °C. The MALDI signal area ratios are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Development of an Affinity Capture MALDI System. Our affinity capture MALDI system was constructed in a stepwise manner, each disk being treated individually, thus simplifying coating, washing, and incubation steps and also preventing cross-contamination between targets, e.g., on a large MALDI sample holder. In addition, the dimensions of the targets made it possible to use very small incubation volumes.

The formation of the SAM was not monitored directly but was confirmed through final antigen capture. The acylation reaction of the DTSP linker with primary amino groups of proteins

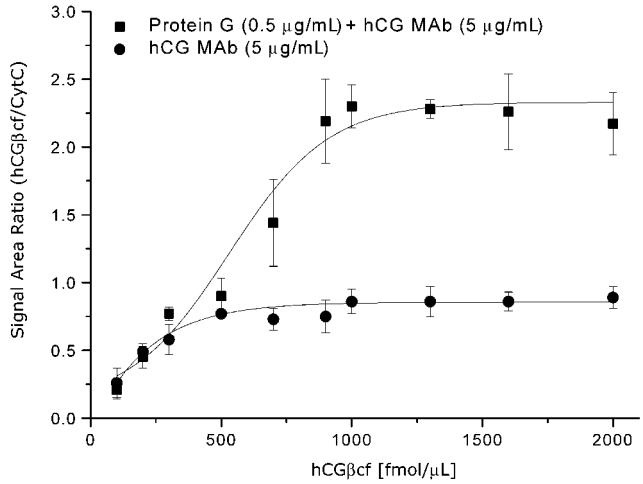

Figure 4. MALDI signal area ratios obtained from random and oriented immunoaffinity disks incubated for 1.5 h at room temperature in 20 μL of hCGβcf (concentrations ranging from 100 fmol/μL to 2 pmol/μL). Antibody orientation increased loading capacity of immunoaffinity disks by ~3-fold. The MALDI signal area ratios are expressed as mean ± SEM ($n = 3$).

competes with the hydrolysis of the active ester (as the acylation is in an aqueous medium). The rate of hydrolysis increases with pH and temperature and decreases with the concentration of primary amine. However, hydrolysis eliminates the need for quenching the active moiety, as an unreacted ester group converts to carboxylic acid. A drawback is that these surface-immobilized carboxylic acid groups may perform as cation exchangers that are capable of retaining highly basic analytes.[14] In addition, adsorption of proteins onto metal surfaces, and gold coatings in particular, is a well-studied phenomenon.[29] Although this problem was not specifically addressed here, the need for washing conditions strong enough to remove nonspecifically bound compounds from the gold surface and the SAM (linker) was taken into consideration. Indeed, MALDI has been used previously as a tool to analyze proteins adsorbed onto target surfaces.[30] A strategy of keeping nonspecific binding to a minimum is important when comparing relative signals in the development of immunoaffinity disks, i.e., optimizing the analysis of just antibody-bound compounds. A washing protocol was developed that involved the use of a detergent. Currently, only few studies have employed detergents for washing affinity capture MALDI supports, i.e., Triton X-100,[6,31] *n*-octyl glucoside,[9,32] or Tween 20,[33] respectively, whereas the majority of other AC MALDI studies used only detergent-free washing buffers.[3,10−15] At the beginning of our investigation, we examined the influence of different concentrations of the nonionic surfactant Triton X-100 on our affinity capture MALDI surface incubated in 10 pmol/μL hCGβcf (Figure 2). A high concentration of hCGβcf was used in order to observe large

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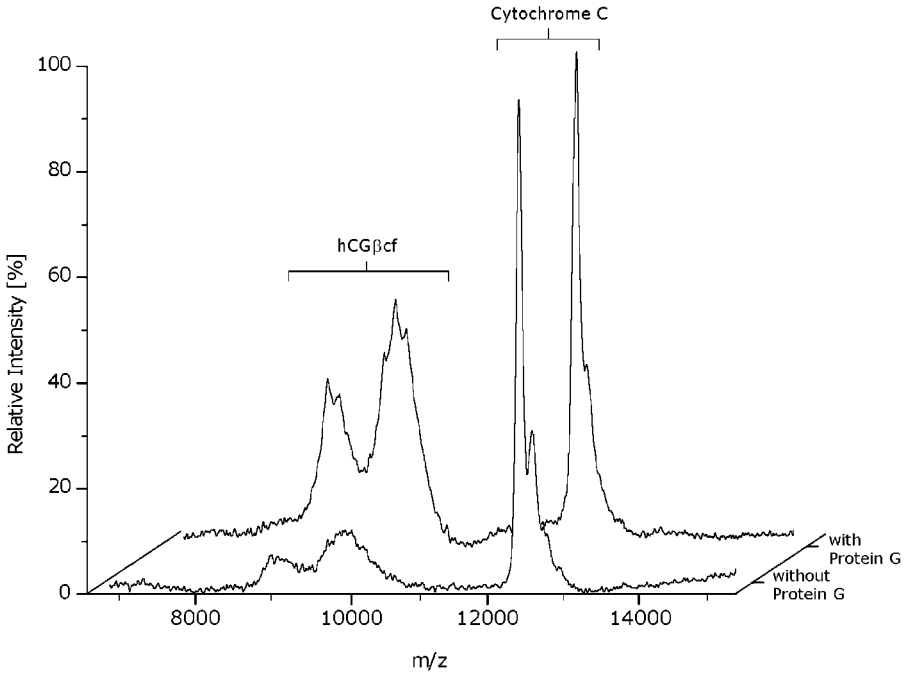

Figure 5. AC MALDI TOF mass spectra of hCGβcf (with cytochrome *c* as an external standard for signal area comparison) using sinapinic acid matrix. Three-dimensional overlay of two spectra of hCGβcf captured from 20 μL of a 1 pmol/μL solution on a surface where hCG MAb (5 μg/mL) was randomly immobilized (foreground) and on a surface where the same antibody was oriented using 0.5 μg/mL protein G (background).

MALDI signal area changes upon washing with detergent, especially for nonspecifically bound hCGβcf. These experiments were carried out in duplicates. A Triton X-100 concentration of 0.4% (w/v) washed away all nonspecifically bound hCGβcf from untreated gold targets (open triangles) or gold targets without linker but with antibody (closed circles); i.e., the signal area ratio (hCGβcf/CytC) was zero. Around this concentration (~0.3−0.5% w/v), only hCGβcf specifically bound to the surface-activated disks (*with antibody*) was measured (closed squares). At a detergent concentration of 1.0% (w/v), the signal area ratio decreased greatly, most probably because of disrupted antibody−antigen interaction. From these data, 0.5% (w/v) Triton X-100 was chosen as the most suitable strength for our washing protocol. The concentration of Triton X-100 chosen in this study was 5 times higher than what was used by others who have incorporated a detergent washing technique in their methodology.[6,31] In general, a rigorous washing of the immunoaffinity disks with the detergent Triton X-100 is acceptable.

In coating "surface-activated disks" with antibody, it is desirable to use a quantity of the antibody that will yield a maximized mass spectrometric response. Too little antibody chosen for surface coating will result in a diminished response, as will too much, paradoxically, because an excessive surface density of antibody can result in diminished antigen-binding efficiency.[21,24,34] To find an optimal antibody concentration for immobilization, immunoaffinity disks were coated with varying concentrations of monoclonal antibody. This resulted in immunoaffinity disks exhibiting different antibody densities on their surfaces. After incubation with antigen, the resulting standardized MALDI signal areas for hCGβcf were plotted against the antibody concentration used for immobilization (Figure 3). Signals from hCGβcf trapped on surfaces with randomly immobilized antibody are shown in the bottom trace. Expectedly, signals increased with higher loading of antibody. However, the MALDI response decreased considerably for higher antibody densities, resulting in a bell-shaped curve. We were able to identify the hCG antibody coating concentration of 5 μg/mL to yield a maximized MALDI response. This phenomenon has also been observed with preparation of immunoaffinity chromatography matrixes, where it was demonstrated that the antigen-binding capacity decreases with increased antibody coupling efficiencies and may be explained by steric hindrance of antibody active sites at higher surface densities preventing interaction with antigen.[34] Indeed, Cooper et al.[35] found that surface crowding of a self-assembled monolayer on gold presenting D-alanine resulted in lower affinity binding of the antibiotic chloroeremomycin as monitored with a surface plasmon resonance biosensor.

The binding capacity of immunoaffinity targets may be attenuated because of inappropriate antibody orientation and steric crowding. Our antibody orientation approach using recombinantly engineered protein G yielded higher MALDI MS readouts for all antibody concentrations analyzed compared to random immunoaffinity disks (Figure 3). All experiments were carried out in triplicates. For the three employed protein G concentrations, 5.0, 1.0, and 0.5 μg/mL, standardized signals intensified with increasing antibody concentrations from 100 ng/mL, reaching a maximum signal at 5 μg/mL, albeit there being very little difference in hCGβcf signals between the protein G concentrations used. The signal amplification for protein G disks in the maximum was ~3-fold compared to those having antibody randomly immobilized. Generally, oriented coupling techniques offer a antigen-binding capacity that is of a factor 2−8 higher than efficiencies obtained

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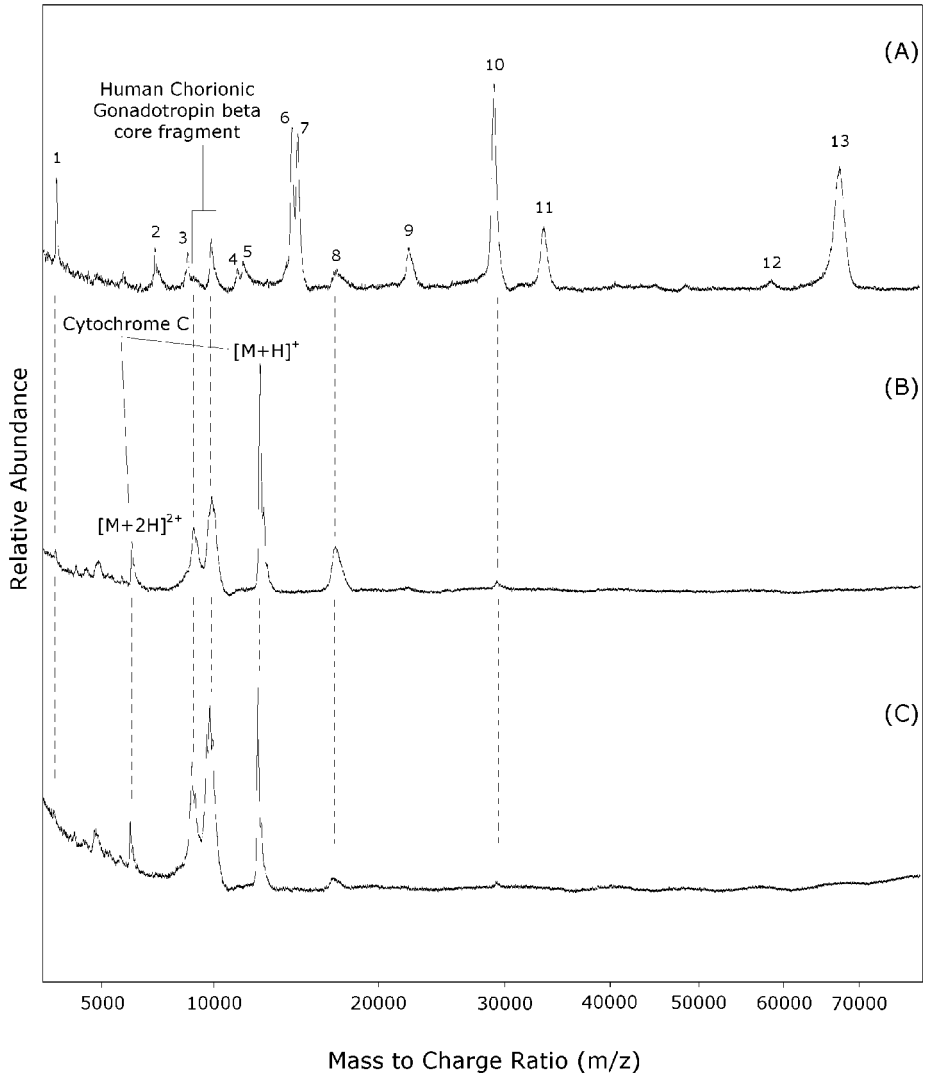

Figure 6. Conventional MALDI (i.e., using a stainless steel target) and affinity capture MALDI analysis of a peptide and protein mixture containing 1 pmol/μl hCGβcf. (A) Conventional MALDI analysis of the protein mixture, (B) hCGβcf capture from the peptide and protein mixture on random (B), and oriented (C) immunoaffinity disks. To compare amounts of captured hCGβcf, the external standard cytochrome *c* was added to (B) and (C). (Note*:* Annotated signals are oxidized insulin chain B (peak 1), for bovine α-lactalbumin (peaks 2 and 6*,* respectively), an unknown contaminant (peak 3), human growth hormone (peaks 4 and 9, respectively), two unidentified polypeptides present in the hCGβcf preparation (peaks 5 and 8*,* respectively), bovine carbonic anhydrase (peaks 7, 10, and 12), and bovine serum albumin (peaks 11 and 13*,* respectively).)

with random coupling methods.[20] For antibody coating concentrations above 5 μg/mL, the following situation was observed: The trace corresponding to the lowest protein G concentration leveled off for the higher MAb concentrations, suggesting free accessibility to all antigen-binding sites on the surface. Here, the protein G was spatially spread out on the surface so that steric crowding was prevented, even for higher antibody concentrations. Hindered accessibility of antigen-binding sites was not observed for this protein G concentration. In fact, the plateau is explained by saturation of surface-immobilized antibodies with hCGβcf. Upon increasing the protein G concentration, the traces descended for the higher antibody concentrations and showed bell shapes. They followed the same pattern as the trace for surfaces with randomly immobilized antibody. As demonstrated here, crowding on solid supports can also be observed with oriented antibodies. Moreover, the binding capacity seems to decrease as the loading of protein G and therefore oriented antibody increases.[21] Generally, a combination of low antibody density and oriented coupling may represent the best approach to prepare a high-capacity affinity capture surface.[34]

The adsorption equilibrium between immobilized antibody and antigen and therefore the performance of the immunoaffinity disk depends on the loading capacity (a function of antibody surface density), antigen concentration, incubation time, pH, temperature, ionic strength, etc.[24] To demonstrate the difference in loading efficiency between random and oriented immobilization, the disks were incubated with increasing concentrations of hCGβcf. Activated disks with or without protein G were coated with 5 μg/mL antibody to give maximized MALDI signals. For oriented immobilization, a protein G concentration of 0.5 μg/mL was employed to minimize steric crowding. Incubation of hCGβcf for 1.5 h at room temperature resulted in curves that plateau at 500 or 1000 fmol/μL hCGβcf incubation concentration for random (closed circles*)* or oriented (closed squares) immobilization, respectively (Figure 4). MALDI signals in the plateau region of the curve were almost 3 times higher for oriented antibody

surfaces. This plateau region of both curves represents surface saturation with antigen. In keeping with this observation, the maximum binding capacity for the oriented immobilized antibody surface was ~3 times higher than for the random immobilized disks (point of inflection of ~900 μmol/L vs ~300 fmol/μL). MALDI surface area ratios for hCGβcf analyzed from 10 pmol/μL using 0.5% (w/v) Triton X-100 on a random MAb surface (Figure 2) are the same as for the plateau region of the bottom trace in Figure 4, which again demonstrates antigen surface saturation. Under the chosen incubation conditions, 100 fmol/μL was the smallest hCGβcf concentration detectable for both types of surfaces. As an adjunct, lower concentrations such as 25 fmol/μL (equates to 250 ng/mL) were routinely observed with both types of surfaces after incubation for 24 h at +4 °C, but further investigations are required to establish the optimum conditions to achieve maximum sensitivity (limit of detection).

The final washing step with purified water removed remnant salts from the surface of the immunoaffinity disk, because salts are likely to diminish the spectral quality and deteriorate resolution.[27] Salt removal leads to increased spectral resolution that is demonstrated by the fact that even for small amounts of captured hCGβcf glycoforms started to resolve. For comparison, only very low spectral resolution was observed for hCGβcf in a previous study on conventional stainless steel targets.[36] On-target sample desalting to improve spectral quality was previously achieved by employing SAM technology to manufacture a C18 surface on a MALDI probe.[37]

To illustrate the difference in MALDI signal intensity, two exemplary spectra generated from a 1 pmol/μL solution of hCGβcf in PBS trapped on a random (5 μg/mL hCG MAb) and oriented surface (0.5 μg/mL protein G; 5 μg/mL hCG MAb) were overlaid (Figure 5). An intensified signal and enhanced spectral resolution can be clearly observed for oriented antibody disks.

Immunoaffinity Capture of hCGβcf from a Test Solution. The performance of the random and oriented immunoaffinity disks prepared was examined on a test mixture containing various peptides and proteins. The conventional MALDI spectrum (on a stainless steel target) of this mixture was recorded from a solution containing no salts as the PBS buffer ions suppress analyte signals (Figure 6A). Apart from the typical hCGβcf doublet signal, the spectrum shows the $[M + H]^+$ for oxidized insulin chain B (peak 1), the $[M + 2H]^{2+}$ and $[M + H]^+$ for bovine α-lactalbumin (peaks 2 and 6, respectively), an unknown contaminant (peak 3), the $[M + 2H]^{2+}$ and $[M + H]^+$ for human growth hormone (peaks 4 and 9, respectively), two unidentified polypeptides present in the hCGβcf preparation (peaks 5 and 8, respectively), the $[M + 2H]^{2+}$, $[M + H]^+$, and $[2M + H]^+$ for bovine carbonic anhydrase (peaks 7, 10, and 12), and finally the $[M + 2H]^{2+}$ and $[M + H]^+$ for bovine serum albumin (peaks 11 and 13, respectively). The signal intensity for hCGβcf was diminished in the presence of the other proteins and peptides. This mixture was incubated on random (Figure 6B) and oriented (Figure 6C) immunoaffinity disks to capture hCGβcf, and following washing, the external standard cytochrome *c* was added with the MALDI matrix in order to compare the amounts captured by both surfaces. For cytochrome *c*, signals for the singly and doubly charged molecular ions were observed, the peak area of $[M + H]^+$ being used for signal area standardization. Both immunoaffinity surfaces were able to capture and enrich hCGβcf on-target and to isolate it from the deliberately added contaminant proteins and peptides, which were either entirely or almost completely removed by washing. An unidentified component was retained (peak 8), suggesting a polypeptide molecule that may be structurally related to hCGβ. Relative MALDI signals for hCGβcf on the oriented antibody surface were again ~3 times higher compared to the random antibody surfaces.

CONCLUSION

The philosophy of using oriented antibodies for on-target capture is equally applicable where newer instruments are employed in an effort to improve analytical sensitivity further. Perhaps more importantly, the availability of immobilized F_c receptors on MALDI targets could have widespread use among those who do not wish to perform surface chemistry, or have laboratory facilities, to link antibodies to MALDI targets. In addition, such pre-prepared targets could allow antibodies to be linked very rapidly, which could have many advantages, e.g., in method development or screening of proteins. Further investigations are now required to study the effect of various biological matrixes, e.g., blood and urine, on the binding affinity of these antibody-oriented disks.

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