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Affinity Capture and Detection of Immunoglobulin E in Human Serum using an Aptamer-Modified Surface in Matrix Assisted Laser Desorption/Ionization Mass Spectrometry

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Abstract

Capture and detection of Immunoglobulin E (IgE) in simple solution and in human serum using an aptamer-modified probe surface for affinity Matrix-Assisted Laser Desorption-Ionization Mass Spectroscopy (affinity MALDI-MS) detection is reported. Detectable signals were obtained for 1 amol of IgE applied either in a single, 1 μL application of 1 pM IgE or after 10 successive, 1 μL applications of 100 fM IgE. In both cases, the surface was rinsed after each application of IgE to remove sample concomitants including salts and free or non-specifically associated proteins. Detection of native IgE, which is the least abundant of the serum immunoglobulins and occurs at sub-nM levels, in human serum was demonstrated and interference from the high abundance immunoglobulins and albumin was investigated. The aptamermodified surface showed high selectivity towards immunoglobulins in serum, with no significant interference from serum albumin. Addition of IgE to the serum suppressed the signals from the other immunoglobulins, confirming the expected selectivity of the aptamer surface towards IgE. Dilution of the serum increased the selectivity toward IgE; the protein was detected without interference in a 10,000-fold dilution of the serum, which is consistent with detection of IgE at amol (pM) levels in standard solutions.

INTRODUCTION

The establishment of proteomic approaches to biomarker discovery and disease profiling in recent years has tested the limits of existing tools for capture and detection of low abundance proteins in biological samples. Affinity binding reagents have played a crucial role in the translation of proteomic discoveries to clinical diagnostics due to their ability to isolate target proteins from complex protein mixtures. Antibodies have been unrivaled as affinity reagents for proteins due to their strong and selective binding; however, drawbacks associated with their production, stability and manipulation have prompted researchers to seek alternatives. Foremost among alternatives are aptamers [1,2], which offer affinity on par with that of monoclonal antibodies, but with important advantages: first, once an aptamer to a target protein has been identified, it can be synthesized, chemically modified and manipulated with ease; second, aptamers are chemically stable and can be reversibly folded and unfolded for capture and release of the target protein, allowing aptamer-modified surfaces to be reused indefinitely. Aptamers have been successfully employed over the past decade in chromatography, capillary electrophoresis, sensing, imaging, and protein isolation and purification [3-7]. A recent addition to the field is the use of aptamer-modified surfaces for affinity protein capture and detection in Matrix-Assisted Laser Desorption-Ionization Mass Spectroscopy (MALDI-MS)

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[8]. In previous work, we demonstrated proof-of-principle of aptamer surfaces for affinity MALDI-MS using the model system of thrombin capture by the G-quartet DNA thrombin-binding aptamer [8]. The approach was subsequently applied in a non-aptameric system of insulin capture from nuclear extracts of cell lysates by a genomic DNA sequence that forms a G-quadruplex [9].

The present work is distinguished from our previous studies of aptamers in affinity MALDI-MS in its focus on the challenging task of detecting a low abundance protein in human serum. Specifically, we describe the capture and detection of Immunoglobulin E (IgE) in human serum using the DNA aptamer to IgE (5′-GGGGC ACGTT TATCC GTCCC TCCTA GTGGC GTGCC CC -3′) [10]. IgE is the least abundant of the immunoglobulins in serum, normally occurring at level of approximately 800 pM [11]. This is 10^5 lower than the most abundant immunoglobulin, IgG, which is normally present at approximately 100 μM in human serum [11]. The IgE aptamer has previously been used for label-free [12,13] and fluorescent-labeled [14,15] detection of IgE in simple solution, providing detectability down to 10^{-10} M IgE (corresponding to 5 fmol using a 50 μL aliquot in the case of one immobilized aptamer sensor [13]). The use of fluorescent-labeled IgE aptamer in affinity capillary electrophoresis gave a detection limit of 46 pM IgE in simple solution, but application to human serum yielded detectable signals only for serum that was spiked with 5 nM IgE and not for native IgE in the serum [16]. In the present work, we achieved capture and detection of native IgE in human serum and found that dilution of the serum by at least 10^3-fold allowed detection of native IgE with little interference from other serum proteins. Detectability compares favorably with the commercial antibody-based ELISA kit (Human IgE ELISA Quantitation Kit, Bethyl Laboratories, Montgomery, TX) that offers 75 pM detection [14].

EXPERIMENTAL

Materials

IgE was obtained from Athens Research (Athens, GA) as the lyophilized protein and reconstituted in deionized water and stored at −4 °C. Human serum albumin (HSA) was obtained from Sigma Aldrich (St. Louis, MO) as the lyophilized powder and stored at 2-8 °C. Human sera (normal and IgA/IgG/IgM free) were obtained from Sigma Aldrich. Standard protein solutions and serum samples were prepared by diluting the commercial protein or serum to the desired concentration in incubation buffer IgE “standard” solutions were prepared by diluting the appropriate volume of the commercial sample in buffer (10 mM sodium phosphate buffer, pH 8.0). For one set of experiments, the IgA/IgG/IgM-free serum sample was further treated to remove albumin using an immunoaffinity kit (ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kit) from Sigma Aldrich.

The DNA oligonucleotides, including the IgE aptamer and a scrambled control sequence (5′-TTTTC CGACC TTCCG GGGGC CCCAG CGTCC TGCAG TG -3′) [10] that does not exhibit affinity towards IgE, were synthesized by Eurogentec (San Diego, CA) with a 5′ thiol modification allowing for attachment to the glass surface. The oligonucleotides were reconstituted in Tris-HCl buffer, pH 7.2, and stored at −4 °C. Other chemicals included 2-(4-hydroxyphenylazo)benzoic acid (HABA), tris(2-4-carboxyethyl)phosphine (TCEP), 3-aminopropyltriethoxysilane (3-APTES), and sulpho-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulpho-SMCC) from Sigma (St. Louis, MO) and DL-1,4-Dithiothreitol 99% (DTT) from Acros (Morris Plains, NJ). All buffers and solutions were prepared using deionized water. Fused silica plates (20mm × 20 mm × 0.75 mm) were made by Valley Design (Westford, MA).

Preparation of DNA Oligonucleotide-Modified MALDI Surfaces

DNA-coated spots were covalently attached to fused silica slides as previously described [8, 9]. The surface of the glass plate was first cleaned and activated by rinsing with methanol, water, and sodium hydroxide. The activated plate was then immersed in a 10% solution of 3-APTES at 100 °C for 4 h, followed by addition of the heterobifunctional linker sulpho-SMCC to the 3-APTES-coated fused silica surface to create spots that were 1-2 mm in diameter. The 5′-thiol modified DNA (aptamer or scrambled control) was treated with TCEP to give a free sulfhydryl group and reacted with the linker at the surface. Finally, the DNA-modified plates were rinsed with buffer to remove excess reagents, dried with ultra-high purity nitrogen, and stored at room temperature.

Affinity MALDI-MS Experiments

Protein capture and detection at the DNA-coated spots on the fused silica plate were performed as follows (unless otherwise specified in the text): One μL sample (either protein standard solution or human serum sample) was applied to each spot and incubated for 30 min. The spots were then rinsed with distilled water for 30 s to remove free or loosely associated proteins, salts and other concomitants, and dried with ultra-high purity nitrogen. One μL MALDI matrix (2.5 mg/ml HABA in 50:50 acetonitrile:water) was then applied to each spot, and the surface dried to crystallization. The fused silica plate was then mounted in a specially designed holder in a Bruker AutoFlex II MALDI-TOF MS system and each spot was analyzed using a nitrogen laser power of approximately 130 μJ. The spot surface was sampled at random at 16.7 Hz in linear mode, collecting 10-12 shots per position, for a total of 400 shots per spectrum.

Following analysis, the fused silica plate was removed and rinsed multiple times, first with 50% acetonitrile in water and then with water, to remove solid matrix and proteins. The plate was then dried with nitrogen and left at room temperature between experiments on the same day, or stored under phosphate buffer in the refrigerator for longer periods. Occasionally, runs were performed between samples in which only matrix was applied to the spots to check for protein carryover from run to run and to check for degradation of the DNA modified surfaces. No evidence of protein carryover or DNA degradation was observed.

RESULTS AND DISCUSSION

IgE Capture and Detection from Standard IgE Solutions

Figure 1 shows results for MALDI-MS of 1 μL of 500 nM IgE solution (500 fmol IgE) on an unmodified stainless steel probe surface (Figure 1A) and at fused silica probe surfaces modified with the IgE aptamer (Figure 1B) and the scrambled IgE control (Figure 1C). The mass spectrum at the aptamer surface exhibits the numerous, multiply charged ions of the 200,000 Da protein that are consistent with the spectrum of IgE at a stainless steel MALDI probe. There is a small amount of non-specific capture at the scrambled control surface. Figure 1D shows the results for a 10,000-fold dilution of the IgE solution (50 amol applied) at an aptamer spot. Interestingly, the spectrum at the lower concentration is dominated by peaks at 66,600, 33,300 and 22,200 m/z, which correspond to the +3, +6 and +9 ions of IgE. This is attributed to dominance of these higher charged ions at the high matrix:protein ratios for these samples, as well as the apparent favorability of ions with multiples of +3 charge. Alternative explanations based on fragmentation of IgE were not borne out by experiments in which IgE was treated with dithiothreitol (DTT) to break the disulfide bonds between the various chains or subjected to high laser powers to aid degradation (results not shown).

The absence of IgE peaks in Figure 2B demonstrates that there is no carry-over of protein between runs and the similarity between the spectra in Figures 2A and 2C demonstrate the reusability of the aptamer spot, which is consistent with our previous work demonstrating

reusability of aptamer spots in MALDI [8]. The remainder of the experiments for this work were performed using and reusing this and several other aptamers spots and control spots on a few different plates, with no evidence of degradation or irreversible alteration, with the exception of spots that were overloaded with serum proteins when concentrated serum was analyzed. Those spots were immediately retired and the remaining spots used.

As in Figure 1D, in Figure 2 only the peaks corresponding to ions with multiples of +3 charge are evident in the spectra at these low levels of IgE. Figures 2D and 2E show the results for 1 amol IgE that was applied in 10 successive incubation/rinse cycles of 0.1 amol IgE (1 μL of 100 fM IgE) prior to application of MALDI matrix and analysis, at an aptamer spot and a scrambled spot, respectively. The results illustrate the ability to preconcentrate IgE at an aptamer spot to achieve detectable levels. The absence of signal for the scrambled spot (Figure 2E) shows that non-specific binding of IgE is insignificant at low concentrations of IgE even after multiple incubation/rinse cycles.

IgE Capture from Commercial Human Sera

Figure 3 shows the results for protein capture from commercial human serum. Undiluted serum on an aptamer spot (Figure 3A) shows several peaks including a series at 150,000, 75,000, 50,000 and 37,500 m/z that is attributed to IgG (150,000 Da) and a series at 67,000, 33,500, 22,200 m/z that may be due to serum albumin (67,500 Da) or the +3, +6, +9 ions of IgE (see below). Analysis of serum that was spiked with IgE by mixing equal volumes of serum and 50 pM IgE solution prior to application to the aptamer spot (Figure 3B) increases the latter peaks, suggesting that the peaks in the spectrum of the unspiked sample are due to IgE. Upon 1000-fold dilution of the serum, the IgG peaks dominate the spectrum at both the aptamer and scrambled spots, showing only small contributions from the IgE peaks at the aptamer spot (Figure 3C) and none at the scrambled spot (Figure 3D). Like the undiluted serum, spiking the diluted sample with IgE suppresses the IgG peaks and enhances the IgE peaks at the aptamer spot (Figure 3E), indicating effective competition by IgE for the aptamer sites. This is further demonstrated in the spectrum of a 10,000-fold dilution of the serum (Figure 3F), which resembles that of low concentrations of IgE with only minor peaks from other proteins.

Figure 4 shows the results for a 10,000-fold dilution of a commercial human serum sample that was treated by the manufacturer to remove IgG, IgA and IgM. In the absence of these abundant immunoglobulins, the aptamer spot (Figure 4A) shows only the peaks for the +3, +6 and +9 ions of IgE that are characteristic of the spectrum for low levels of the protein (see Figure 1D). The scrambled spot shows no evidence of protein capture (Figure 4B). Spiking the sample with IgE increases the peaks at the aptamer spot (Figure 4C), supporting their identification as IgE peaks. Again, there is no evidence of protein capture at the scrambled spot (Figure 4D).

Studies of Serum Albumin

Although the results in Figure 4 support the assignment of the peaks at 67,000, 33,500 and 22,200 m/z to IgE, further experiments were performed to determine whether serum albumin contributed to these peaks as well. Albumin must be considered as a potential source of nonspecific interference in any analysis of serum proteins due to its presence at mM concentrations in normal serum.

Figure 5 shows the spectra of 10 pmol albumin at a stainless steel MALDI surface (Figure 5A) and 1 pmol albumin an aptamer spot (Figure 5B). Signals were not detected at aptamer or scrambled spots for albumin at or below fmol levels, which corresponds to nM concentration (results not shown). Both Figures 5A and 5B exhibit peaks at 66,700 m/z (+1 ion of albumin) and at the m/z corresponding to the +2 and +3 ions. These peaks coincide closely with the

peaks for the +3, +6 and +9 ions of IgE. The peak at 133,000 m/z is attributed to a singly-charged albumin dimer formed in the MALDI process, and coincides with a small peak that is observed at high IgE concentration on stainless steel (Figure 1A) but not on the DNA-coated spots for any concentration of IgE. The small peak at 44,000 m/z corresponds to the +3 ion of the albumin dimer and is present in the both of the albumin spectra (Figures 5A and 5B) but never in the IgE spectra.

The absence of peaks at 133,000 and 44,000 m/z in the spectra of human serum and IgA/IgG/IgM-free serum support the assignment of the peaks at 67,000, 33,500 and 22,200 m/z for those samples to IgE rather than albumin. This assignment is supported by capture experiments using a 1000-fold dilution of the IgG/IgA/IgM-free serum that was treated to remove albumin. The results are shown in Figures 5C and 5D for an aptamer spot and a scrambled spot, respectively. The peaks at 44,000 and 133,000 m/z are not present in either spectrum, confirming that these peaks can be used as specific indicators of albumin capture. Their absence from all of the spectra for the serum capture experiments indicates that albumin does not interfere with the aptamer-based affinity MALDI-MS determination of IgE.

In order to determine the effect of incubation time on IgE capture and potential interference from albumin, aliquots containing 50 fmol each IgE and albumin were analyzed at two aptamer spots, one after 3 min incubation and the other after 30 min incubation as in the other experiments in this work. After 3 min (Figure 5E) the peaks at 66,600, 33,300 and 22,200 m/z dominate the spectrum, with minor peaks at 100,000 and 50,000 m/z that correspond to the +2 and +4 ions of IgE that are expected at this relatively high IgE concentration. These peaks become more intense after a 30 min incubation, but there is no sign of the albumin peaks at 133,000 and 44,000 m/z, confirming that only IgE is captured and/or detected at the aptamer surface. The expanded regions in Figures 5E and 5F also show development of the IgE parent peak at 200,000 m/z in going from 3 min (Figure 5E inset) to 30 min incubation (Figure 5F inset), further confirming identification of the captured protein as IgE.

CONCLUSIONS

The results demonstrate capture and detection of IgE at the amol level from pM solutions of the protein. Successive incubation/rinse cycles were used to achieve detection of IgE at concentrations of 100 fM. Other, more abundant immunoglobulins, notably IgG, interfere with IgE capture from serum at the IgE aptamer-modified surface, but there is no evidence of significant interference from non-immunoglobulin proteins such as albumin. Dilution of the serum sample greatly reduces the response to non-IgE immunoglobulins. Since IgE normally occurs at levels of 800 pM in human serum [11], its detection in 10^3-10^4-fold dilutions of serum is consistent with the detection of 1 pM-100 fM IgE in standard IgE solutions.

While MALDI-MS does not yet match fluorescence for quantitative measurements, our results demonstrate the ability of the affinity MALDI-MS aptamer platform to capture and detect a low abundance protein in serum, to preconcentrate the protein directly at the probe surface, and to confirm the identity of the affinity-captured protein through examination of the mass spectrum. The reusable aptamer-modified surfaces could easily be adapted to microarray formats and the application/incubation/rinse protocols are amenable to automation.

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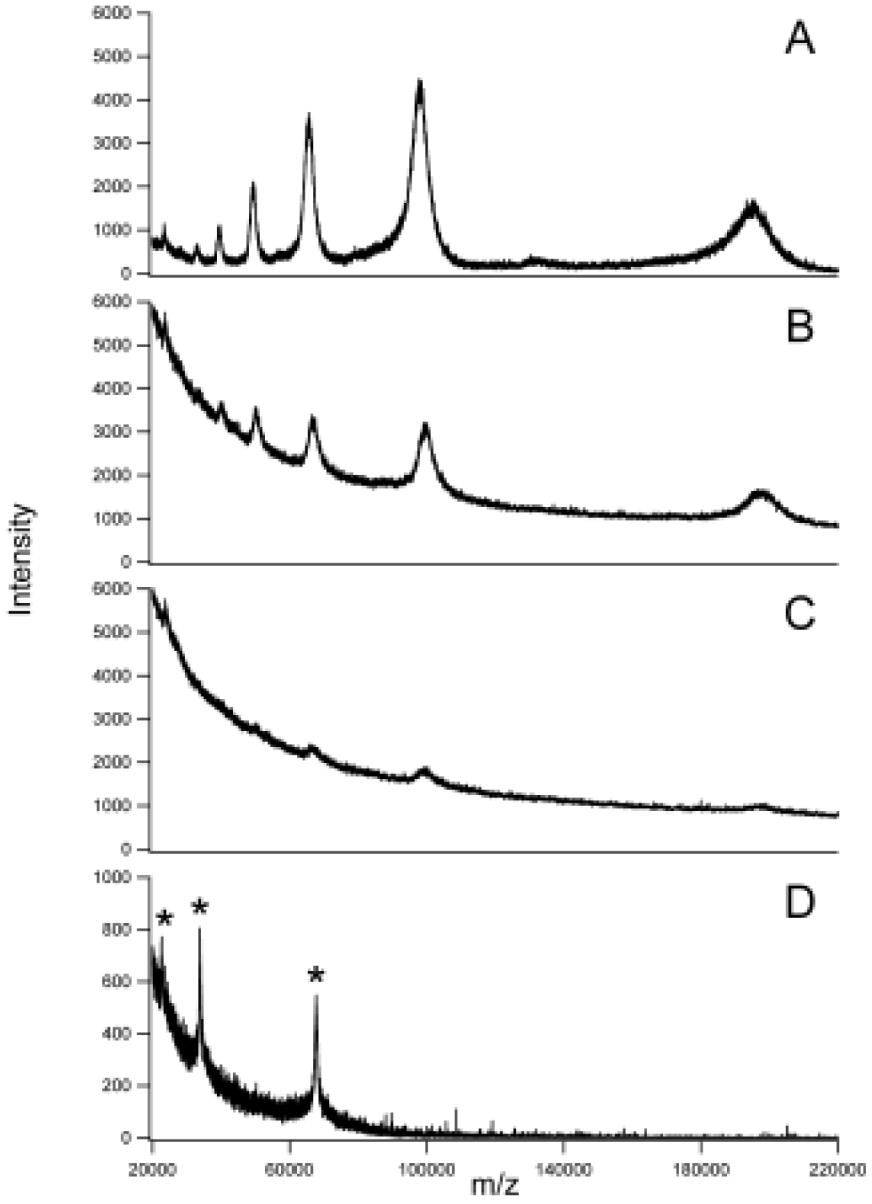

Figure 1.

Mass spectra of IgE at various surfaces. (A) 500 fmol IgE on unmodified stainless steel probe surface; (B) 500 fmol IgE on aptamer spot; (C) 500 fmol IgE on scrambled spot; (D) 50 amol IgE on aptamer spot; asterisks denote peaks at 22,200, 33,500, and 67,000 m/z, corresponding to +9, +6 and +3 ions of IgE. Note that the intensity scale of A cannot be compared with that of B-D.

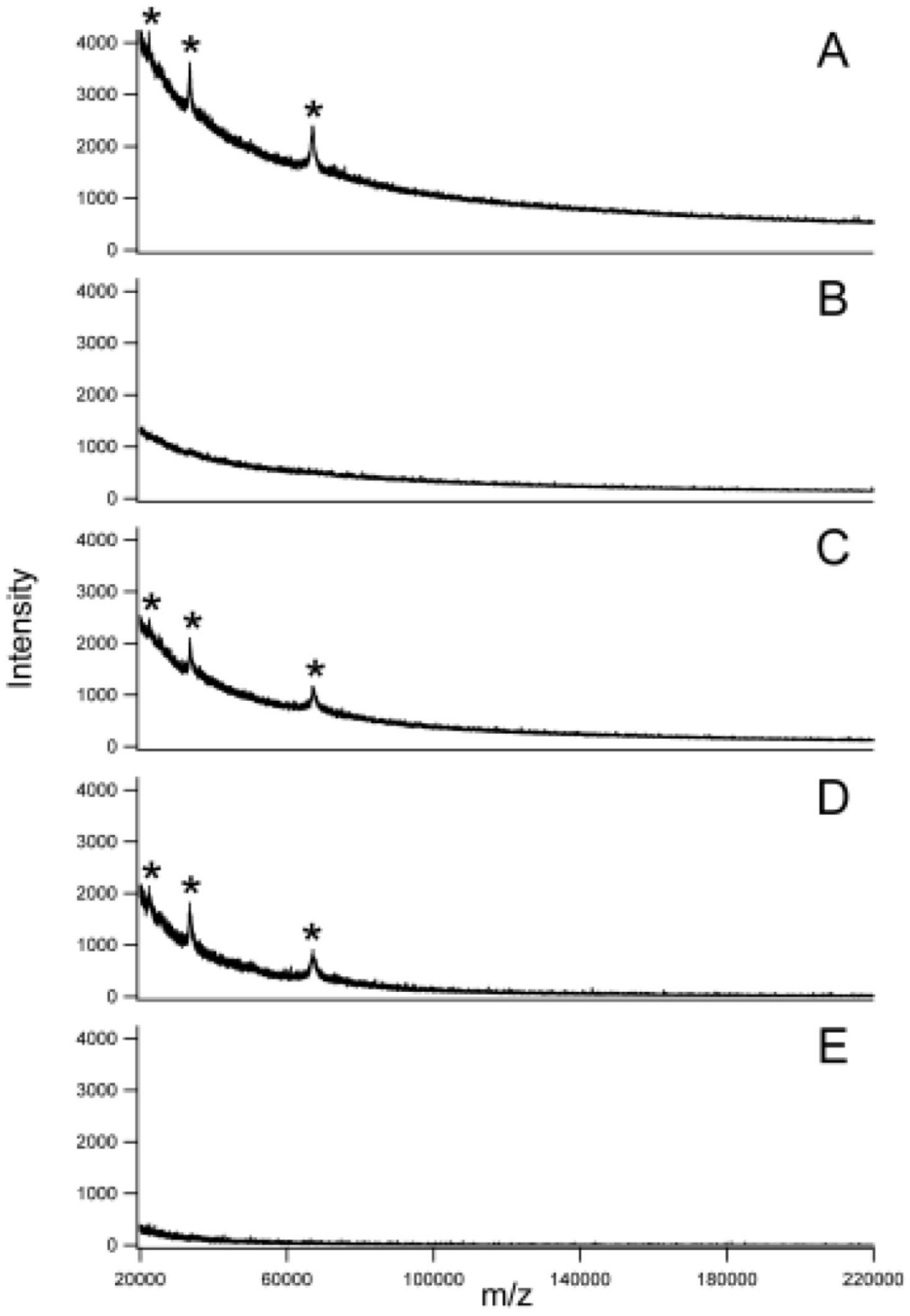

Figure 2.

Capture and preconcentration of IgE. (A) First trial of 1 amol IgE (1 μL of 1 pM IgE) on aptamer spot (Run 1) (B) Blank run with matrix only at same spot after rinsing to remove matrix and protein from Run 1; (C) Second trial of 1 amol IgE at same spot (Run 2); (D) 1 amol IgE on aptamer spot, applied in 10 successive incubation/rinse cycles, each of 0.1 amol IgE (1 μL of 100 fM IgE) prior to application of MALDI matrix; (E) 1 amol IgE (1 μL of 1 pM IgE) on scrambled spot. Asterisks denote peaks at 22,200, 33,500, and 67,000 m/z, corresponding to +9, +6 and +3 ions of IgE.

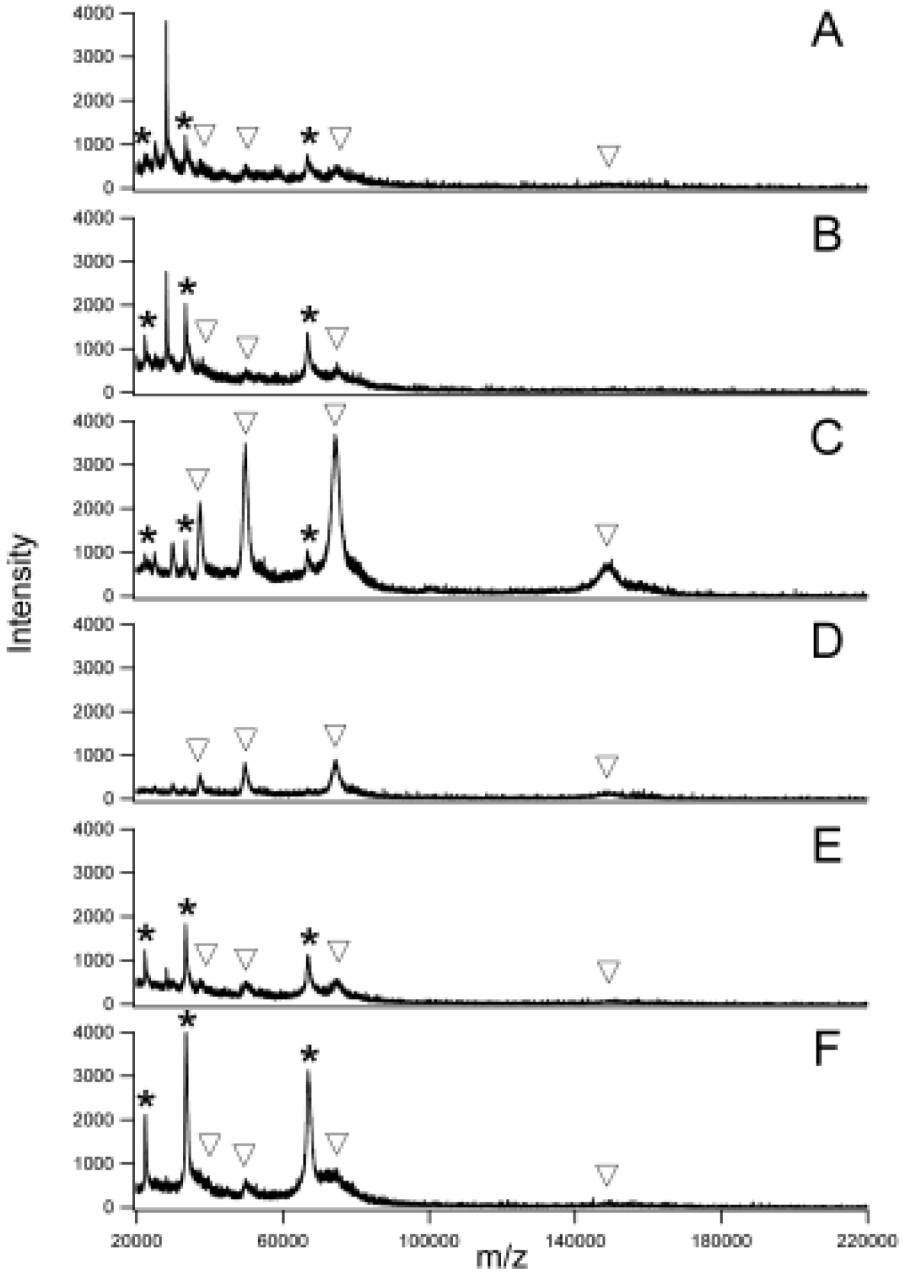

Figure 3.

Capture of IgE from human serum. (A) Undiluted serum (1 μL) on aptamer spot; (B) 50:50 dilution of serum with 50 pM IgE on aptamer spot (1 μL 50% serum with 25 amol added IgE); (C) 1000-fold dilution of serum (1 μL) on aptamer spot; (D) same as (C) on scrambled spot; (E) 1000-fold dilution of serum further diluted 50:50 with 50 pM IgE on aptamer spot (1 μL 2000-fold diluted serum with 25 amol added IgE); (F) 10000-fold dilution of serum further diluted 50:50 with 50 pM IgE on aptamer spot (1 μL 20000-fold diluted serum with 25 amol added IgE). Asterisks denote peaks at 22,200, 33,500, and 67,000 m/z, corresponding to +9, +6 and +3 ions of IgE. Inverted triangles denote peaks at 37,500, 50,000, 75,000 and 150,000 m/z, corresponding to +4, +3, +2 and +1 ions of IgG.

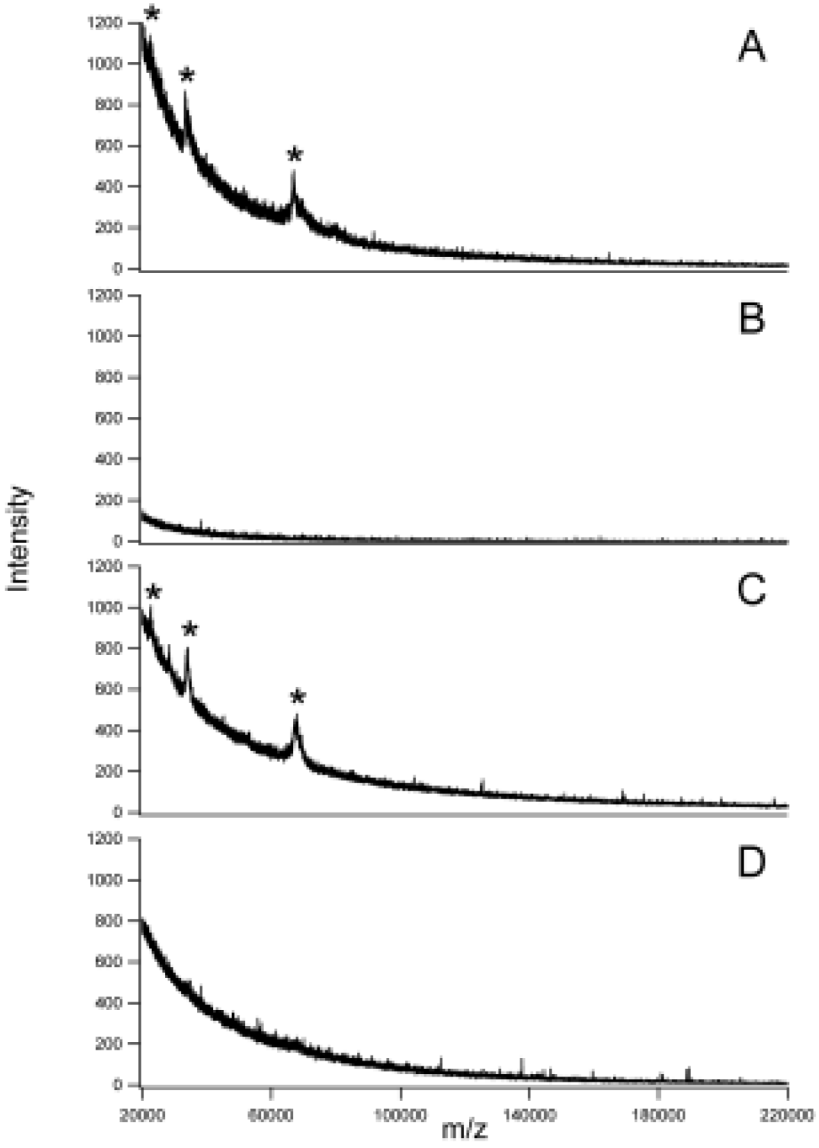

Figure 4.

Capture of IgE from IgG/IgA/IgM-free human serum. (A) 10000-fold dilution of IgG/IgA/IgM-free serum (1 μL) on aptamer spot; (B) same as (A) on scrambled spot; (C) 10000-fold dilution of IgG/IgA/IgM-free serum further diluted 50:50 with 50 pM IgE on aptamer spot (1 μL 20000-fold diluted serum with 25 amol added IgE); (D) same as (C) on scrambled spot. Asterisks denote peaks at 22,200, 33,500, and 67,000 m/z, corresponding to +9, +6 and +3 ions of IgE.

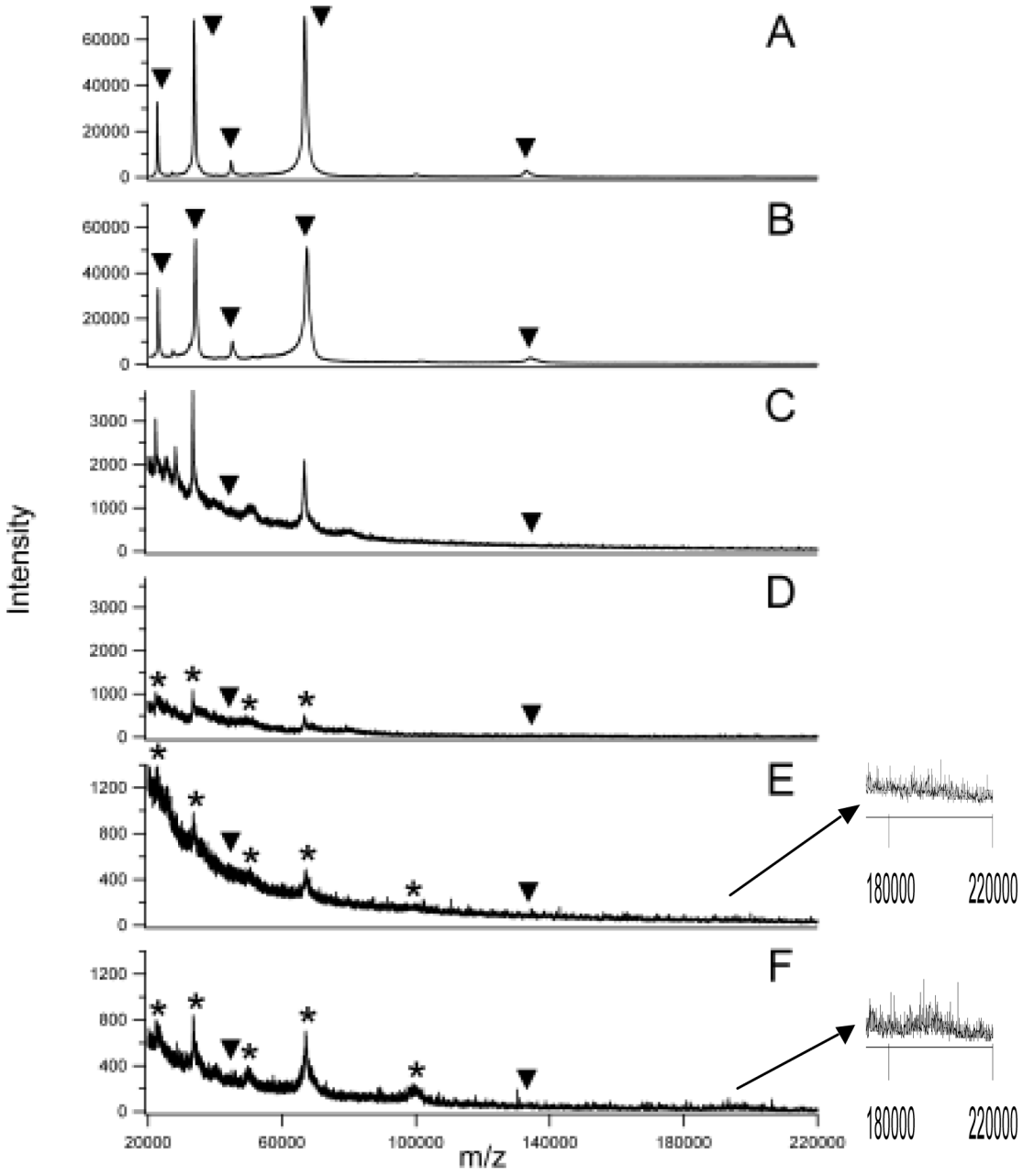

Figure 5.

Capture of human serum albumin and IgE. (A) 1 pmol albumin (1 μL of 1 μM albumin) on unmodified stainless steel probe surface; (B) same as (A) on aptamer spot; (C) 1000-fold dilution of IgG/IgA/IgM-free serum further treated to remove albumin (1 μL) on aptamer spot; (D) same as (C) on scrambled spot; (E) 50 fmol each IgE and albumin at aptamer spot after 3 min incubation; (F) same as (E) after 30 min incubation. Asterisks denote peaks at 22,200, 33,500, 50,000, 67,000 and 100,000 m/z, corresponding to +9, +6, +4, +3, +2 ions of IgE. Inverted solid triangles denote peaks at 22,000, 33,250, and 66,700 m/z, corresponding to +3, +2, +1 ions of albumin, and at 44,000 and 133,000 m/z, corresponding to +3 and +1 ions of albumin dimer. Arrows in (E) and (F) point to expanded views of area around IgE parent peak at 200,000 m/z.