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Surfactant-Enhanced Desorption/Ionization on Silicon Mass Spectrometry

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Perfluorinated surfactants are demonstrated to dramatically enhance desorption/ionization on fluorinated silicon (DIOS) mass spectrometry. Perfluorooctanesulfonic acid improved the signal-to-noise ratio of tryptic digests and gave a 3-fold increase in the number of peptides identified. Similar results were also obtained using perfluoroundecanoic acid ; yet among the seven different surfactants tested, controls such as nonfluorinated sodium dodecyl sulfate or fluorinated molecules with minimal surfactant activity did not enhance the signal. The same surfactants also enhanced the DIOS-MS signal of amino acids, carbohydrates, and other small organic compounds. The signal enhancement may be facilitated by the high surface activity of the perfluorinated surfactants on the fluorinated silicon surfaces allowing for a higher concentration of analyte to be absorbed.

Surfactants are characterized by their ability to form dynamic aggregates or micelles[1] and constitute an important chemical platform in biological sample preparation and analysis. For example, sodium dodecyl sulfate (SDS) has gained widespread use within protein sample preparation for extraction,[2] solubilization of hydrophobic proteins,[3] and foremost for its use in SDS-polyacrylamide gel electrophoresis (PAGE).[4] Unfortunately, surfactants generally have a negative effect on matrix-assisted laser desorption/ionization (MALDI)[5−7] and electrospray ionization (ESI);[8,9] therefore, numerous approaches to remove surfactants prior to mass spectrometry (MS) analysis have been developed.[10−12] Also, strategies to recover MALDI-MS signal from SDS-containing samples have evolved;[7,13−15] for example, Zhang et al.[13,14] utilized a two-layer MALDI plate deposition technique with a bottom layer consisting of HCCA matrix and the protein−SDS sample as top layer to improve MALDI-MS spectra. Similarly, employing an ion-pairing reagent incorporated into the bottom layer, Rajnarayanan and Wang[15] recovered the MALDI signal from SDS-containing samples. Jensen et al. obtained MALDI spectra from protein samples containing SDS up to 0.2% as long as pH was kept below 2 with TFA.[7] An acid-labile surfactant (ALS)[16] approach was developed by Ross et al., where the ALS will decompose during acidic conditions and hence does not interfere to the same extent as SDS with MALDI-MS. Very recently, a further development of cleavable detergents for use in MALDI-MS was introduced by Norris and co-workers.[17] They prepared a combined detergent/matrix molecule that acts as a detergent in solution, and upon application on the MALDI target, acid is added and the detergent/matrix is cleaved to become matrix.[17] Another alternative to SDS was suggested by Zhang and Liang.[18] They employed ammonium dodecyl sulfate (ADS) for PAGE separation and MALDI-MS analysis of some proteins and observed that ADS gave better MALDI-MS results than SDS.[18]

Although some nonionic detergents i.e., *N*-octylglucoside, previously have proven to enhance MALDI-MS response of some peptides,[19] polymers,[20] and integral membrane proteins,[21] Amado and co-workers were first to show that the negative effect of SDS in MALDI-MS can be reversed with SDS concentrations above 0.3%.[22] They obtained useful spectra with SDS concentrations as high as 10% and concluded that micelle formation of the surfactant could be connected to the signal recovery.[22] The concept of surfactant-aided MALDI[23] was further introduced by Breaux et al., where they found that the MALDI-MS analysis of hydrophobic

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peptides gained signal-to-noise ratios (S/N) by addition of SDS (>0.5%) to a sample mixture. Tummala and Limbach illustrated that the addition of 0.1−0.3% SDS to tryptic digests of different proteins prior to MALDI-MS analysis resulted in an increase in the number of tryptic peptides detected, thereby increasing the sequence coverage when attempting to identify the proteins through peptide mass fingerprinting.[24] Chen and Tsai used the surfactant *p*-toluenesulfonic acid to enhance the surface-assisted laser desorption/ionization-MS signal of methylephedrine and other small organic molecules.[25] The surfactant cetrimonium bromide was used by Gou and co-workers to suppress matrix background[26] for small-molecule analysis with MALDI-MS.

Perfluorinated alkyl surfactants have alkyl chains that are both hydrophobic and olephobic[27] (oil repelling); the perfluorinated amphiphiles are more surface active and more hydrophobic than their corresponding hydrogenated analogues in terms of lowering of the interfacial tension and critical micelle concentration.[28] Furthermore, Ishihama et al. demonstrated that perfluorinated surfactants were more compatible with ESI-MS than other surfactants.[29] Perfluorinated carboxylic acids have found use as ion-pair reagents for reversed-phase separations of amino acids[30,31] and small peptides with ESI-MS detection;[32] they have also found application in micellar electrokinetic chromatography directly coupled to mass spectrometry.[33] Fluorinated surfaces have further been used for surface-induced dissociation in conjunction with plasma desorption mass spectrometry.[34,35] A novel method for evaluation of oligomeric protein structures using perfluorooctanoic acid as detergent in conjunction with PAGE was developed by Ramjeesingh et al., perfluorooctanesulfonic acid-PAGE (PFOS-PAGE).[36] The usefulness of this strategy has subsequently been illustrated in several publications.[37−39]

Desorption/ionization on silicon (DIOS),[40] developed in our laboratory, has previously been illustrated as a versatile platform for high-sensitivity detection of small molecules and peptides,[41,42] and the effect of derivatizing the DIOS surface with different silylation reagents has been investigated.[42] In the present study, we have investigated the use of perfluorinated surfactants to enhance DIOS-MS analysis of tryptic peptides and small molecules. It is advantageous to use perfluorinated surfactants for sample preparation as they have proven to be very useful as ion-pair reagents for reversed-phase separations and as detergents in conjugation with PAGE. Therefore, they would be very useful in sample preparation applications if they could be successfully combined with MS analysis. Herein, we report a clear correlation between the use of perfluorinated surfactants and enhanced DIOS-MS signal for both small molecules and peptides. The potential of using surfactants as signal enhancers for mass spectrometry and the insight it provides into the mechanism of desorption for DIOS is also discussed.

EXPERIMENTAL SECTION

Chemicals. Methanol and tetrahydrofuran (THF), both Optima grade, as well as hydrofluoric acid 47−51% were obtained from Fisher (Fair Lawn, NJ). Other solvents were ethanol 200 proof from AAPER (Shelbyville, KY) and water produced in a NANOpure system Barnstead International (Dubuque, IA). The surfactants used in the study were obtained through and used without any further purification: perfluorooctanesulfonic acid potassium salt (PFOS, >97%; Fluka, Buchs, Switzerland), perfluoroundecanoic acid (PFUnA, 95%; Sigma, St. Louis, MO), 3-(perfluorodecyl)propane-1-ol (PFDOH; Fluorous Technologies Inc., Pittsburgh, PA), and SDS (99%; Sigma). Proteins bovine serum albumin (BSA) 99% and α-casein 70% were both obtained from Sigma. The carbohydrates Galβ1−4GlcNAcβ-Sp, Galβ1−3GalNAcβ-Thr, Neu5Acα2−6GalNAcα-Thr, and Fucα1−2Galβ1−4GlcNAcβ1−3Galβ1−4GlcNAc−Sp were kindly provided by Dr. Ola Blixt at the Carbohydrate Synthesis/Protein Expression Core of The Consortium for Functional Glycomics (La Jolla, CA). For the derivatization of the DIOS chips (heptadecafluoro-1,1,2,2-tetrahydrodecyl)dimethylchlorosilane from Gelest, Morrisville, PA) and chlorodimethyloctadecylsilane (95%; Aldrich) were used. Digestions were performed using trypsin with specific activity of >5000 units/mg (Promega, Madison, WI). All other chemicals/compounds were obtained in high purity from Sigma-Aldrich. Stock solutions of surfactants were prepared before every experiment. Stock of PFUnA (100 mM) was prepared in 100% methanol, PFOS (50 mM) was prepared in 99.7% methanol/0.3% H_2O, and PFDOH (100 mM) was prepared in 80% methanol/20% THF. From these stock solutions, working solutions were prepared in different concentrations (0−25 mM) in 90/10 methanol/water. These were subsequently used to dilute the stock solutions of protein digests and small molecules.

DIOS Chip Preparation. A detailed description of DIOS chip preparation have been published elsewhere.[43] Briefly, pieces (∼3.5 × 3.5 cm) were cut out from low-resistivity (0.005−0.02 Ω·cm) n-type silicon wafers (500−550-μm thickness) from Silicon Quest International (Santa Clara, CA). The silicon chips were washed with ethanol and dried under N_2 gas before being placed on top

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of a gold foil (anode) in a Teflon chamber. A platinum wire (cathode) was placed in the cavity, and the chamber with the chip in the bottom was filled with 10 mL of 25% HF in ethanol (v/v). The silicon chip was subsequently electrochemically etched (1 min) with a current of 5.6 mA under white light illumination from a fiber-optic light source hosting a 250-W quartz/halogen lamp (model I-250, CUDA Fiberoptics) and placed above a transparent film with a printed pattern and a series of lenses focusing the pattern on to the chip.[43] The printed "gradient" pattern was made in Adobe Photoshop (San Jose, CA) by fitting 10 × 10 circles (covering ~2 × 2 cm) made with a gray scale gradient light to dark or vice versa using the radial toolbar function, while the area surrounding the circles was made black. The mask was subsequently printed on a standard laser printable transparency film. This created a black mask with circles through which the light could pass, causing a photopattern (spots) on the chip as it was etched. Another set of chips were prepared using a mask consisting of 10 × 10 completely black circles on an otherwise transparent surface. This caused an "inverse" type of etch, in which the boundary of the spots were defined by the coherently black photopatterned area around them. In this manner, the spots are actually just indirectly photopatterned by scatter light and thus very lightly etched. After etching, the chips were rinsed with ethanol and dried under N_2 gas.

The H-terminated porous silicon surface was next oxidized by exposure to ozone (flow rate 0.5 g/h) from an ozone generator Expotech (Huston, TX) for 30 s. Immediately after oxidation, the chip was placed in a glass Petri dish and its surface was covered with 100 μL of (heptadecafluoro-1,1,2,2-tetrahydrodecyl)dimethylchlorosilane for the C_8F_{17} derivatization. Chlorodimethyloctadecylsilane dissolved 15% (w/v) in toluene was used for the C_{18} derivatization. With a larger Petri dish used as a lid, the silylation reaction proceeded at 90 °C for 30 min (6 h for C_{18}), after which the chip was taken out, thoroughly rinsed with methanol (while still hot), and dried under a stream of N_2 gas before being fitted onto a modified MALDI target (Applied Biosystems) with an adhesive tape. No further modifications were made to the chips prior to their use.

Protein Digest Preparation. BSA, 1 mg/mL (in H_2O), was boiled for 1 h and 100 μL was taken out; 37.5 μL of 10 mM dithiothreitol (DTT) in 25 mM NH_4HCO_3 buffer was added and the resultant mixture incubated at 60 °C for 30 min. After cooling, 17 μL of 55 mM iodoacetamide in 25 mM NH_4HCO_3 buffer was added and the resultant mixture incubated at room temperature for 30 min (in the dark). Afterward, 75 μL of the DTT solution was added and sample was left at room temperature for 30 min. This was followed by addition of 33 μL of trypsin (0.1 μg/μL), 1:30 enzyme-to-protein ratio, and the digestion was incubated for 16 h (37 °C) after which the reaction was stopped with 3 μL of acetic acid. The α-casein was digested without reduction−alkylation as follows; to a 100-μL α-casein aliquot, from 1 mg/mL stock in 40:60 ACN/H_2O, 750 μL of 2 M urea in 25 mM NH_4HCO_3 was added followed by 20 μL of trypsin (0.1 μg/μL). The digestion was incubated at 37 °C 16 h. A second addition of 20 μL of trypsin (0.1μg/μL) was added, and the sample was incubated another 16 h at 37 °C. The digested proteins were diluted to 200 nM with appropriate surfactant working solution, resulting in 100 fmol deposited on spot (0.5 μL).

Small Molecules. A stock solution containing verapamil (2.5 μM), bistacrine, propranolol, haloperidol (5 μM), atenolol (10 μM), adenosine 5′-monophosphate, tryptophan, lysine, prednisone (1 mM), caffeine, phenylalanine (2 mM), and histidine (300 μM) was prepared in 80:20 H_2O/methanol. This solution was subsequently diluted 10 times with the appropriate working solution of surfactant; 0.5 μL was spotted on the DIOS chip. To the final solution, 0.1% HCl was added. A stock solution of carbohydrates was prepared containing the following; sucrose, maltotriose, Neu5Acα2−6GalNAcα-Thr, Fucα1−2Galβ1−4GlcNAcβ1−3Galβ1−4GlcNAc−Sp (250 μM), and Galβ1−4GlcNAcβ-Sp, Galβ1−3GalNAcβ-Thr (50 μM). The stock solution was diluted 10 times with the appropriate working solution of the surfactant of which 0.5 μL was spotted on the DIOS chip.

Mass Spectrometry. All DIOS-MS/MALDI-MS measurements were performed on an Applied Biosystems MALDI-TOF STR in the reflectron mode. The samples were irradiated with a 337-nm nitrogen laser operated at a repetition rate of 15 Hz. An acceleration voltage of 25 000 V was employed, and delayed extraction periods of 50−250 ns was used for optimal resolution. The lowest possible laser energies to achieve analyte signal were consequently used. The digest spectra were calibrated using internal peptide, and peptide mass fingerprints were searched against Swiss-Prot using Mascot (MatrixScience Ltd.). Mascot matches peptides according to the MOWSE algorithm.[44] The following search parameters were used: a peptide tolerance of ±200 ppm, a missed cleavage of 1, and mammalian taxonomy. Spectra processing was performed using Data Explorer (Applied Biosystems) and included baseline correction, noise filtering, and monoisotopic peak list filtering.

Calculations. To illustrate a more comprehensive S/N compromising several peptides, a "summed" or cumulative S/N value was computed. This S/N was calculated by summation of the ion count obtained using Data Explorer (Applied Biosystems) for the following tryptic peptides: SEIAHR (m/z 712), YLYEIAR (m/z 927), LGEYGFQNALIVR (m/z 1479), KVPQVSTPTLVEVSR (m/z 1639), and RHPYFYAPELLYYANK (m/z 2045). The peptide summed ion count was then divided by the average ion count for the m/z interval 2900−3000, resulting in a "summed" S/N value that accurately represented the visual impression of the spectra. The average S/N values displayed in Figure 4 were calculated from at least three individual spectra.

Safety Considerations. Extreme care should be taken in handling hydrofluoric acid solutions because of their toxicity and corrosiveness. All inhalation, ingestion, or skin or eye contact should be strictly avoided. Etching of silicon wafers should be conducted in a ventilated fume hood using proper double-layered nitrile gloves, lab coat, and goggles. Hydrofluoric acid solution spills and burns can be neutralized and treated with 2.5% calcium gluconate gel.

RESULTS AND DISCUSSION

Surfactant-Enhanced Peptide and Small-Molecule DIOS-MS. Perfluorinated surfactants used in this study include perfluorinated carboxylic acid with different chain lengths (C_8, C_9, C_{10}, C_{11}), PFOS, which is structurally similar to SDS, and PFDOH. The fluorinated alcohol was selected to compare the

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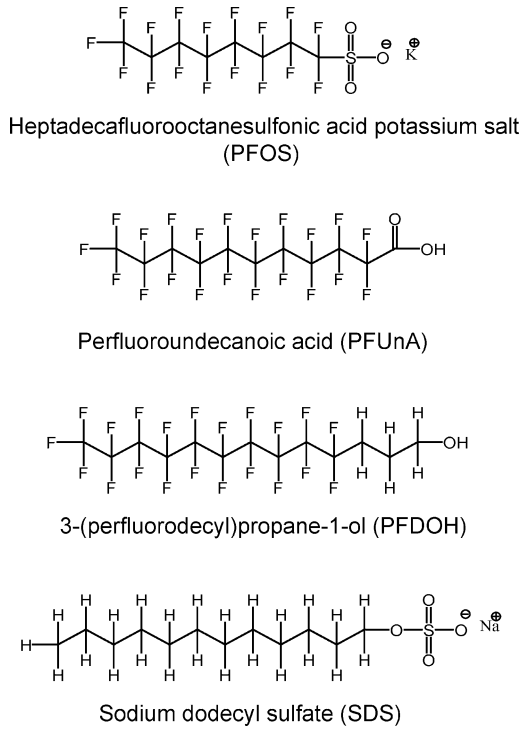

Figure 1. Structures of investigated surfactants.

effect of the polar headgroup (Figure 1), and sodium dodecyl sulfate served as a nonfluorinated control. Among the surfactants investigated in this study, PFOS displayed the most pronounced signal enhancing effect (Figure 2). Among the perfluorinated carboxylic acids, PFUnA (C_{11}) gave the best result (data not shown).

DIOS-MS experiments were performed on protein model systems (BSA and α-casein tryptic digests, 100 fmol) and on a variety of small molecules to illustrate the effectiveness of perfluorinated surfactants in enhancing the DIOS signal. A general observation in using perfluorinated surfactants as additives in DIOS-MS analysis was a reduction of 10−50% in minimum laser energy threshold required to yield signal. Typical DIOS-MS data of a 100-fmol BSA digest mixed with four different surfactants at a concentration of 0.25 mM are shown in Figure 2A−D. In Figure 2E, the same digest analyzed without any surfactant is displayed. An enhancement of signal was observed with PFUnA and PFOS whereas PFDOH and SDS did not affect the appearance of the spectra to any significant extent. From the data shown in Figure 2A, the addition of 0.25 mM (∼0.01%) PFOS to the digest increased the number of detected peptides from 16 to 52 and the protein sequence coverage from 24 to 62% compared to a digest without surfactant (Figure 2E). Interestingly, the surfactant PFOS by itself only yielded one peak at m/z 576 corresponding to its potassium adduct. Some of the unstarred peaks in Figure 2A are suspected to be [peptide − H + PFOS + Na + K]$^+$ and [peptide + Na/K]$^+$ adducts. A 2-fold increase in the number of detected peptides and sequence coverage was achieved with PFUnA (Figure 2B) as compared to only H_2O (Figure 2E). Yet the increase in signal intensity was not as pronounced as with PFOS. The perfluorinated alcohol, PFDOH, did not yield any signal enhancement compared to H_2O (Figure 2C and E). From the comparison of spectra in Figure 2D and E, the addition of SDS (0.25 mM, ∼0.007% w/v) did not affect appearance of the spectra

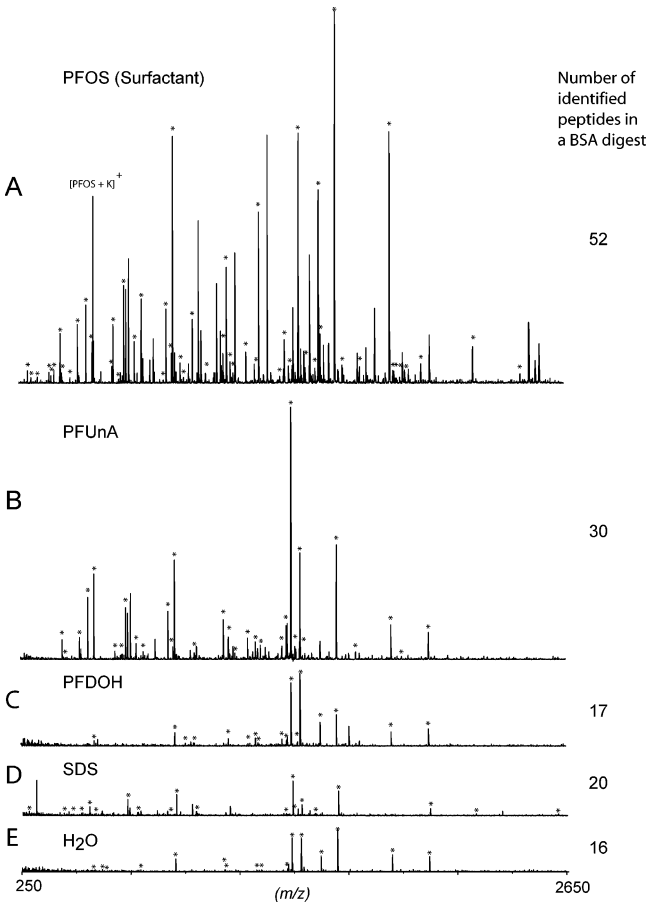

Figure 2. DIOS mass spectra of a tryptic digested BSA (100 fmol) with different surfactants at a concentration of 0.25 mM. The asterisk (∗) indicates Mascot-identified proteolytic peptides. Some of the nonstarred peaks in spectra A and B likely come from [peptide − H + surfactant + Na + K]$^+$ or [peptide + Na/K]$^+$. *Y*-intensity scale is the same in (A−E). (A) PFOS; (B) PFUnA; (C) PDFOH; (D) SDS; (E) water control.

to any significant degree. The observation that hydrocarbon chain surfactant does not achieve the same signal-enhancing effect as its perfluorinated counterpart is also illustrated in Table 1 (both C_8F_{17} and C_{18} functionalized surfaces). PFUnA and SDS did not yield any significant background signal under investigated experimental conditions.

Since the successful implementation of DIOS-MS for the analysis of small organic molecules is well established,[41−43] we also explored how perfluorinated surfactants affected the DIOS-MS signal of a variety of organic molecules with mass ranging from 100 to 1000 Da (Figure 3). A DIOS-MS spectrum of a mixture consisting of small molecules analyzed with and without PFOS on a C_8F_{17} functionalized chip is displayed in Figure 3A. The small molecules were detected as [M + H]$^+$at lower laser energies (0.7−1.0 μJ) including, lysine (m/z 147), histidine (m/z 156), phenylalanine (m/z 166), caffeine (m/z 195), tryptophan (m/z 205), propranolol (m/z 260), atenolol (m/z 267), adenosine 5′-monophosphate (m/z 348), prednisone (m/z 359), haloperidol (m/z 376), verapamil (m/z 455), and bistacrine (m/z 493). At higher laser energies, sodium adducts were observed for phenylalanine at m/z 188 and prednisone at m/z 381. It should be mentioned that propranolol, haloperidol, verapamil, and bistacrine appeared in the spectrum when laser energy was increased (>0.7

Table 1. Evaluation of DIOS-MS. BSA Digest Data for Two Derivatized DIOS Surfaces and Two Added Surfactants

	DIOS surface					
	C_8F_{17}			C_{18}		
surfactant	PFOS	SDS	H_2O	PFOS	SDS	H_2O
sequence coverage (%)	65 ± 5	38 ± 14	30 ± 1	27 ± 5	33 ± 4	26 ± 4
no. of identified peptides	55 ± 3	29 ± 9	21 ± 2	25 ± 4	29 ± 2	20 ± 4
Mascot score value[a]	358 ± 29	105 ± 56	67 ± 21	83 ± 27	104 ± 19	55 ± 16

[a] Mascot returns a score value for each search performed with the MOWSE[42] algorithm. The score value is defined as $-10 \times \mathrm{LOG}(P)$, where P is the absolute probability of the peptide match being a random event. A probability of 10^{-20} then becomes a score of 200.

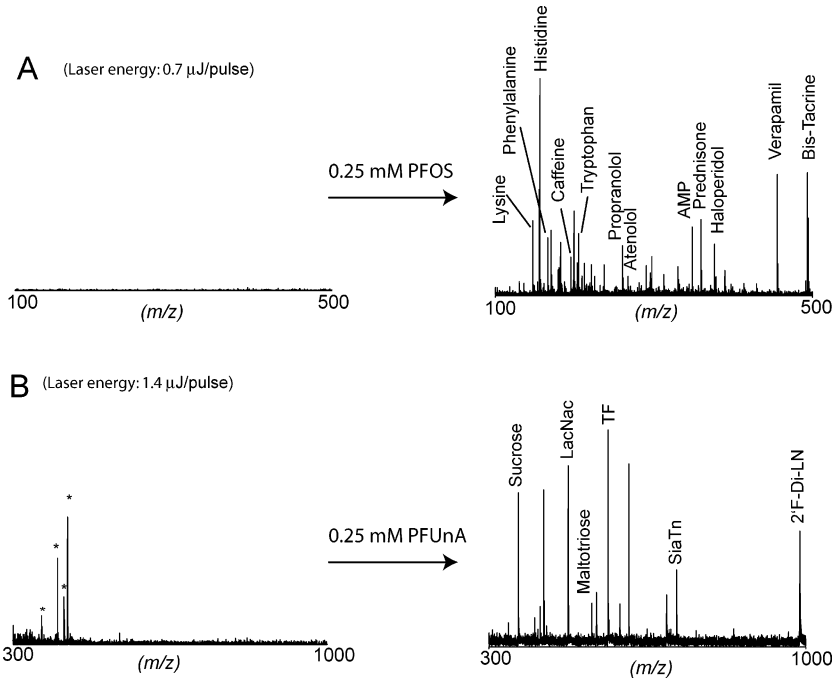

Figure 3. (A) Small molecules analyzed with and without 0.25 mM PFOS on an "inverse" chip (see Experimental Section). Propranolol, verapamil, and bistacrine showed up without surfactant if the laser energy was increased. (B) Carbohydrates analyzed with and without 0.25 mM PFUnA on "gradient" chip. Trivial names used are LacNac (Galβ1−4GlcNAcβ-Sp), TF (Galβ1−3GalNAcβ-Thr), SiaTn (Neu5Acα2−6GalNAcα-Thr), and 2′F-Di-Ln (Fucα1−2Galβ1−4GlcNAcβ1−3Galβ1−4GlcNAc−Sp). Asterisk (∗) indicates background ions originating from the DIOS surface. Intensity scale is the same for both without and with the respective surfactant.

μJ), but when laser energy used in Figure 3A (0.7 μJ) was applied to the samples without surfactant, no signal was detected (Figure 3A). A series of carbohydrates (sucrose m/z 365, Galβ1−4GlcNAcβ-Sp m/z 475, maltotriose m/z 527, Galβ1−3GalNAcβ-Thr m/z 563, Neu5Acα2−6GalNAcα-Thr m/z 714, and Fucα1−2Galβ1−4GlcNAcβ1−3Galβ1−4GlcNAc−Sp m/z 986) used in this study were detected as $[M + Na]^+$. Removing the surfactant from the sample mixture caused analyte signal to disappear (Figure 3B).

Using perfluorinated surfactants, the range of DIOS-amenable analytes can be expanded and the sensitivity is enhanced. Interestingly, we did not find any molecule that ionized on DIOS, for which the surfactant actually lowered the sensitivity or hampered the measurement.

Signal Enhancement. To quantitatively investigate the surfactant effect on DIOS-MS, a cumulative S/N (see Experimental Section) of five peptides from a BSA digest mixed with different surfactant concentrations was calculated in the observed mass range (Figure 4). For both PFOS and PFUnA, a significant increase in S/N was observed in the concentration range 0.25−2 mM (Figure 4 PFOS and PFUnA), whereas the S/N continuously deteriorated with higher concentrations of SDS and completely

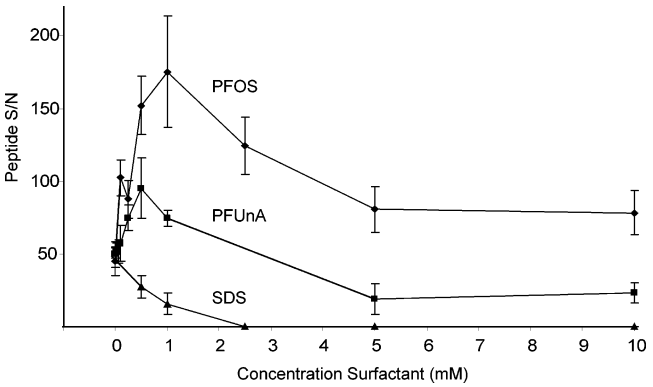

Figure 4. Peptide S/N with different surfactants plotted against various surfactant concentrations. Symbols: diamonds (PFOS), squares (PFUnA), and triangles (SDS). See Experimental Section for calculation of S/N values.

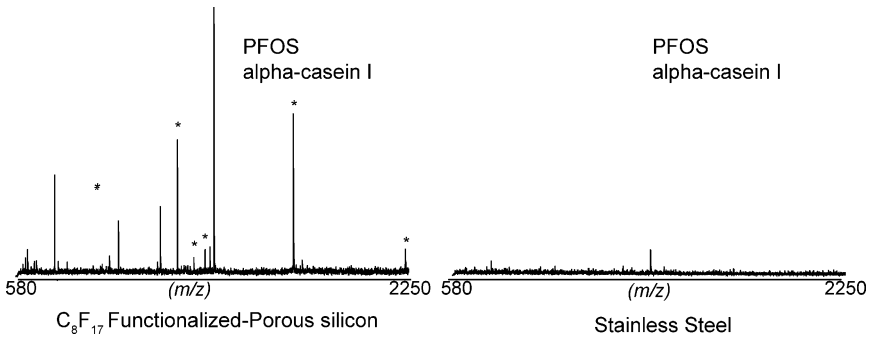

Figure 5. α-Casein digest analyzed with PFOS (0.25 mM) on a C_8F_{17} (F17) functionalized DIOS surface and a stainless steel plate (MALDI target). Asterisk (∗) in the left spectrum indicates Mascot-identified proteolytic peptides. The perfluorinated surfactant did not display any appreciable UV absorption at 337 nM.

disappeared above 1 mM (Figure 4 SDS). It is worth noting that PFOS gave a maximum effect at ∼1 mM, yet continued to be beneficial up to a concentration of 25 mM.

The enhanced signal generated from the perfluorinated surfactants required that they be tested to determine whether they were acting as MALDI matrixes. In general, a MALDI matrix[45] has the ability to absorb at the laser energy and to facilitate desorption/ionization. The perfluorinated surfactants investigated in this study did not display any appreciable UV absorbance at 337 nm on a UV spectrophotometer. Further analysis of an α-casein digest with the surfactant on a stainless steel MALDI-plate (Figure 5) generated no signal. Both experiments supported the idea that the perfluorinated surfactants were not acting as matrix material.

The use of surfactants in MALDI-MS has recently received more attention. Work performed by Amado et al.[22] illustrated that peptide signal that initially decreased by addition of SDS could be recovered as the concentration of SDS approached the critical micelle concentration (cmc). Breaux et al.[23] showed that hydrophobic peptides gained MALDI-MS signal intensity with addition of SDS. Furthermore, the importance of MALDI sample preparation and choice of the surfactant as well as choice of matrix was proven to greatly affect the MALDI signal.[13] Recently, Tummala and Limbach[24] showed that SDS can enhance the sequence coverage of tryptic digest analysis above a certain SDS concentration. Zhang and Li[14] also showed that some proteins gained more in sequence coverage than others by addition of SDS after digestion, prior to MS analysis. From these studies, it is clear that the successful combination of surfactants with MALDI depends on the type of protein and surfactant, when the surfactant was added and how the MALDI sample is prepared.

However, since matrix crystal formation is not a critical step in DIOS-MS, our data cannot be applied to this mechanism. Further, DIOS-MS experiments at cmc values for the surfactants (SDS[46] (∼8 mM), PFOS[47] (8 mM, at 80 °C), PFUnA[48] (0.43 mM, sodium salt at 60 °C)) gave no S/N improvement (Figure 4). This provided further evidence that our results do not support a general cmc-dependent mechanism of signal enhancement as previously suggested for MALDI-MS.[22−24]

Based on the results obtained from the different surfactant structures (Figures 1-4), the ionizable headgroup and the fluorinated carbon backbone appear to be important structural features for successful use of surfactants in DIOS-MS. These structural features also support the view that surfactant interactions with analytes as well as its interaction with the surface are important to the observed signal enhancement. It is well known that the physical properties of fluorinated amphiphiles offer unique characteristics such as high surface activity and lowering of surface tension compared to those of their hydrogenated analogues.[27,28] Additional PFOS and SDS experiments on the C_8F_{17} and C_{18} functionalized DIOS surface (Table 1) yielded significantly more peptide ions with the combination of C_8F_{17} functionalized surface and PFOS. These data further suggest that the interaction between the analyte and the perfluorinated surfactant, and the subsequent interaction between the perfluorinated surfactant and the fluorinated surface, is important for the observed enhancement.

Explosive vaporization[49−51] refers to the mechanism through which top layers of solvent molecules on a surface are ejected in response to rapid heating. It is suspected that the solvent−surface wetting is an important feature in DIOS[52] and that residual solvent adsorbed on the pSi surface is integral to the desorption/ionization process;[43,52] consequently, explosive vaporization has been proposed as a possible desorption/ionization mechanism for DIOS.[40]

The results presented in this article are consistent with this mechanism, where the perfluorinated surfactant is deposited onto the porous silicon (pSi) surface, which is functionalized with fluorine−carbon chains. Layers or clusters of surfactant interacting with the C_8F_{17} functionalized surface allow the analyte and solvent molecules to be trapped on the surface of the pSi. Since the surfactants spread evenly over the surface, a uniform distribution of surfactant/analyte mixture on the surface is achieved. This also results in a uniform mass spectral data acquisition across the surface. The data in Table 1 indicate that perfluorinated surface combined with perfluorinated surfactant generates signals with the highest S/N and most comprehensive display of molecules present; further, the perfluorinated surfactant offers a significant

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advantage over their alkane counterparts in terms of signal quality. Methanol/water clusters trapped in pores between layers could further facilitate desorption/ionization through more efficient energy transfer. This view is further supported by the observation that samples spotted with perfluorinated surfactant require significantly lower laser energies to yield signal.

CONCLUSION

Surfactants are very important in sample preparation, but unfortunately, they have proven difficult to combine with high-sensitivity mass spectrometry analysis. The perfluorinated surfactants employed in our study have previously found a wide range of applications in separation science as ion-pairing agents and in protein sample preparation as analogues to SDS. Therefore, our findings might have implication for a more successful combination of surfactants in biological sample preparation/separation combined with mass spectrometry detection.

For small molecules and peptides, we have observed a significant signal-enhancing effect of perfluorinated surfactants using DIOS-MS combined specifically with perfluorinated carboxylic acid (C_{11}) and perfluorinated octanesulfonic acid (C_8). These surfactants significantly improved the S/N and increased the number of peptides observed. The mechanism behind the observed phenomenon remains to be elucidated, yet the ionizable headgroup and the perfluorinated backbone of the surfactant appear to promote surfactant−analyte−surface interactions, potentially enhancing explosive vaporization.

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