Quantitative Determination of the Peptide Retention of Polymeric Substrates Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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Polymer surface–peptide binding interactions have been shown previously to lead to reductions in peptide matrix assisted laser desorption/ionization (MALDI) ion signals. In previous studies, increases in surface–peptide binding were characterized by the increases in both the initially adsorbed and retained quantities of ^{125}I-radiolabeled peptides. The present studies establish a specific correlation between the peptide retention properties of the polymer surface and the reduction in the peptide MALDI ion signal. This correlation is demonstrated by obtaining MALDI mass spectra of angiotensin I applied to various polymer surfaces having a range of peptide adsorption and retention properties. In addition, the use of a MALDI based method of standard additions is shown to allow the quantitation of the polymer surface–peptide retention affinity for angiotensin I and porcine insulin. The MALDI standard additions method for measurement of surface–peptide retention affinities offers a number of significant advantages over conventional radiolabeled peptide binding methods and promises to be a valuable tool for the determination of this important biomaterial characteristic. (J Am Soc Mass Spectrom 2000, 11, 62–68) © 2000 American Society for Mass Spectrometry

Substantial research has been devoted to the development of new biomaterials with various surface–protein binding properties. The desired biomaterial protein binding properties can vary dramatically depending upon the specific application envisioned. For example, a given application may require a biomaterial ideally having low to negligible binding affinities for all proteins (e.g., contact lenses), whereas other applications may require biomaterials having high binding affinities for all, or a very specific subset, of proteins. Ultimately, the surface–protein binding affinity of a given biomaterial can have considerable implications for the biocompatibility of that material [1–3]. As such, analytical methods for the determination of surface–protein binding affinities for existing or newly developed biomaterials are of considerable importance.

A common method for the determination of surface–protein binding affinity involves the exposure of a biomaterial to a solution containing ^{125}I-radiolabeled proteins [4, 5]. Two measurements of protein binding affinity may be determined using this approach. The first measurement is of the quantity of protein that adsorbs to a surface with sufficient strength to resist rinsing with a buffer solution. The second measurement is of the quantity of protein that is retained by a surface following exposure of the adsorbed protein coated biomaterial to a desorbing agent, e.g., surfactants, solutions of high ionic strength, etc. The partial reversibility of protein adsorption to a given surface has been used to infer the existence of populations of both weakly surface-bound and strongly surface-bound proteins and may be indicative of the effects of surface–protein orientation, surface–induced protein denaturation, protein–protein interactions, etc. [6, 7]. Thus, surface–protein adsorption and retention affinities are important characteristic properties of a given biomaterial.

In previous studies we have shown that peptide MALDI ion signals are directly influenced by surface–peptide binding interactions. In these studies surface–peptide binding affinity was quantitated by measurement of the adsorption and retention affinities of the surfaces for ^{125}I-radiolabeled peptides. Essentially, it was found that as the surface–peptide adsorption and retention affinity increased the peptide MALDI ion signal decreased—presumably because of poor incorporation of the peptide in the MALDI matrix [8]. This

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inverse relationship has also been shown to be relatively uninfluenced by the method of sample preparation, at least to the extent that a variety of "conventional" sample preparation methods also reflect an influence of surface–peptide binding affinity on peptide MALDI ion signals [9]. In our previous studies, however, no effort was made to correlate the reduction in peptide MALDI ion signal with the peptide adsorption or retention affinity of the polymer surface. This correlation could not be made because of the similar relative adsorption and retention properties of the amine modified polymer surfaces used in those studies.

In the present studies MALDI mass spectra of angiotensin I applied to a variety of unmodified polymer surfaces are recorded and compared with the adsorption and retention affinities of these surfaces, as reflected in the binding of ^{125}I-radiolabeled angiotensin I. Examination of the trend in angiotensin I MALDI ion signal with changes in the binding affinity of the polymer surfaces demonstrates that the reduction in the peptide MALDI ion signal is specifically correlated with the surface–peptide retention affinity. Furthermore, the data suggest that, as the quantity of peptide applied to the surface is decreased to the point where it approaches the quantity retained by the surface, the peptide MALDI ion signal approaches zero. Based on this observation, a standard additions approach is evaluated as a method for the direct determination of surface peptide retention affinity. The results demonstrate that for angiotensin I and porcine insulin applied to a variety of conventional polymer materials, surface–peptide retention affinities determined by the MALDI standard additions method are in good agreement with those determined by the more conventional radiolabeled peptide binding approach.

Experimental

Chemicals and Materials

Sheets of the polymers low density poly(ethylene) (LDPE, 0.25 mm thickness), poly(etheretherketone) (PEEK, 0.125 mm thickness), poly(etherimide) (PEI, 0.25 mm thickness), poly(ethersulfone) (PES, 0.125 mm thickness), poly(ethylene terephthalate) (PET, 0.125 mm thickness), poly(tetrafluoroethylene) (PTFE, 0.10 mm thickness), and poly(vinylidene difluoride) (PVDF, 0.25 mm thickness) were obtained from Goodfellow (Berwyn, PA). Angiotensin I, porcine insulin, and α-cyano-4-hydroxycinnamic acid (ACHCA) were obtained from Sigma and used without further purification. ^{125}I-radiolabeled peptides were provided by the University of Texas Southwestern Medical Center (Dallas, TX). Phosphate buffered saline (PBS) was obtained from Sigma and prepared as directed to achieve a concentration of 10 mM phosphate and a solution pH of 7.4. Sodium dodecyl sulfate (SDS) and trifluoroacetic acid (TFA) were obtained from Sigma and prepared by dilution of the pure substances with distilled water to achieve final concentrations of 0.3% (w/v) and 10% (v/v), respectively.

^{125}I-radiolabeled Peptide Adsorption to Polymeric Substrates

The peptide binding affinity of the commercially available polymers was determined by exposure of the materials to ^{125}I-radiolabeled angiotensin I and porcine insulin. 1.0 cm $\times$ 2.0 cm samples of each of the various polymers (LDPE, PEEK, PEI, PES, PET, PTFE, and PVDF) were placed in a flow through cell with a total internal volume of 20 mL. The PBS solution was introduced into the flow through cell and the polymer samples were allowed to equilibrate for 3 min. The PBS solution was then flushed from the cell by the introduction of 30 mL of either [^{125}I]angiotensin I or [^{125}I]porcine insulin (both 0.010 mg/mL in PBS, 1.0 μCi/mL). The peptide solution was allowed to contact the polymer samples for 20 min after which it was displaced by introduction of 50 mL of PBS solution. Finally, the flow through cell was drained and the polymer samples removed.

The adsorption affinity of the polymer for the ^{125}I-radiolabeled peptide, either angiotensin I or porcine insulin, was determined by placing the polymer samples in test tubes and measuring the radioactivity using a well-type gamma counter (Wallac 1282, Compugamma). Conversion of the measured radioactivity to a quantity of peptide was accomplished by comparison of the measured radioactivity to the radioactivity of 200 μL of the 0.010 mg/mL ^{125}I-radiolabeled peptide solution. Subsequently, 4 mL volumes of 0.3% SDS solution were added to each test tube and the samples were incubated for 3 min with intermittent shaking. The samples were then removed to fresh test tubes and the radioactivity recounted in order to determine the retention affinity for the ^{125}I-radiolabeled peptides. Quantitation of the retained protein was determined using the procedure described above.

MALDI Mass Spectrometric Analysis

All MALDI mass spectrometric analyses were performed using methods previously described [8]. In brief, a 0.48-cm diameter disk (0.18 cm^2) of a given polymer was secured to a stainless steel probe tip using commercial adhesive. Stock solutions of angiotensin I or porcine insulin dissolved in aqueous PBS at concentrations of 1.0 mg/mL were prepared and diluted with additional aqueous PBS as needed. Subsequently, a 2 μL aliquot of the desired concentration peptide solution was applied to the polymer disk and allowed to dry under ambient conditions (drying time ~45 min). Following drying of the peptide solution, 2 μL each of ACHCA in methanol (15 mg/mL) and 10% TFA in distilled water were co-deposited on the peptide coated polymer surface and allowed to dry. In all cases, it was

Table 1. Adsorption / retention of [^{125}I]angiotensin I to polymer substrates and the *x* intercepts of the MALDI standard additions plots

	Adsorbed [^{125}I]angiotensin I[a]			Retained [^{125}I]angiotensin I[a]			MALDI *x* intercept[c]	
Polymer	ng/cm^2	molecules/cm^2	Coverage[b]	ng/cm^2	molecules/cm^2	Coverage[b]	ng/cm^2	R^2
PEEK	86 ± 4	4.0×10^{13}	64%	18 ± 3	8.5×10^{12}	14%	16.8 ± 7.2	0.963
PTFE	130 ± 30	6.0×10^{13}	97%	12 ± 3	5.7×10^{12}	9.1%	11.5 ± 5.6	0.970
PEI	46 ± 9	2.1×10^{13}	34%	8.5 ± 2.6	3.9×10^{12}	6.3%	8.3 ± 3.3	0.989
LDPE	71 ± 18	3.3×10^{13}	53%	8.2 ± 3.6	3.8×10^{12}	6.1%	7.6 ± 2.7	0.995
PET	34 ± 5	1.6×10^{13}	25%	6.8 ± 2.7	3.2×10^{12}	5.1%	7.1 ± 2.8	0.993
PES	51 ± 13	2.4×10^{13}	38%	6.7 ± 2.0	3.1×10^{12}	5.0%	7.5 ± 2.4	0.995
PVDF	19 ± 4	0.88×10^{13}	14%	5.2 ± 1.6	2.4×10^{12}	3.9%	5.4 ± 2.4	0.995

[a]Polymer surface areas are calculated from macroscopic dimensions and do not account for surface microscopic morphological structure. The values shown are the average quantity of peptide present on five samples. The error corresponds to one standard deviation of the data set.
[b]Percent coverage calculated using a cross sectional area for angiotensin I (1.6×10^{-14} cm^2) calculated from the reported volume of insulin [10] and by assuming linear scaling of the peptide volume with molecular mass.
[c]A minimum of six surface concentrations were used to generate the standard additions plot. At each surface concentration a total of 20 MALDI mass spectra were acquired randomly from three samples. The error in the *x* intercept was calculated according to [17].

noted that the peptide containing solution and/or the matrix/TFA solutions came in contact with the entire polymer surface at some time during the sample preparation process. Thus, opportunity for the entire polymer surface to interact with the peptide solution occurred either during initial peptide solution deposition or via diffusion during the matrix/TFA solution deposition.

Peptide MALDI mass spectra were taken on a home built linear time-of-flight mass spectrometer using 337 nm radiation. In all cases a standard protocol was used for the acquisition of the peptide MALDI mass spectra. In this protocol the desorption laser intensity was adjusted in real time using a variable neutral density filter to achieve the largest protonated peptide ion signal while maintaining baseline resolution between the $[M + H]^+$ and $[M + Na]^+$ ion signals. In general this laser intensity was approximately 2× threshold for ion formation. After maximizing the protonated peptide ion signal a 20 shot MALDI mass spectrum was acquired without further adjustments of the laser intensity. Cumulatively, a total of twenty 20 shot mass spectra were acquired randomly from three samples of each peptide/polymer combination. Subsequently, the singly protonated peptide ion signals were integrated and the average and standard deviation determined.

Results and Discussion

Correlation of Reduction in Peptide MALDI Ion Signals with Surface-Peptide Retention Affinity

Initially, the adsorption and retention affinities of a variety of commercially available polymers for [^{125}I]angiotensin I were determined using the methods described. The values determined are given in Table 1. The adsorption affinity of the polymer reflects the quantity of peptide which interacts with the polymer surface with sufficient strength to resist removal during flushing of the flow through cell with PBS. The retention affinity of the surface reflects the quantity of peptide which is strongly bound to the polymer surface and is able to resist removal by vigorous washing with the surfactant SDS. This latter value is thought to be indicative of the strength of the surface–peptide interaction.

Also given in Table 1 are the number of molecules of angiotensin I adsorbed or retained per macroscopic unit area and the percent surface coverage that results from these adsorption and retention values. These values should be viewed as upper limit approximations only, because the actual microscopic surface area is unknown, i.e., surface morphological features would be expected to increase the microscopic surface area depending on the roughness of the surface. In addition, the cross sectional area of angiotensin I has been estimated from the reported cross sectional area of insulin [10] by assuming linear scaling of the peptide volume with molecular mass. Despite these approximations it is clear that in no case is more than a monolayer of angiotensin I initially adsorbed to the polymer surface and that less than 15% of the surface is covered after washing the surface with SDS.

For the group of polymers evaluated it should be noted that there is no strong correlation between the angiotensin I adsorption and retention affinities of the polymer surfaces, i.e., PTFE has the highest adsorption affinity, but only retains 9% of the angiotensin I, while PEEK, with the next highest adsorption affinity, retains 21% of the angiotensin I. The varied adsorption and retention affinities of the group of polymers allowed the correlation between the surface-angiotensin I adsorption or retention affinity and the reduction in peptide MALDI ion signal to be investigated.

In the first group of experiments the quantity of angiotensin I applied to each polymer surface was held constant at a value chosen to exceed by approximately 10% the quantity adsorbed by the polymer with the highest adsorption affinity (PTFE). Specifically, the stock 1.0 mg/mL solution of angiotensin I was diluted to 0.013 mg/mL and 2.0 μL aliquots of this solution

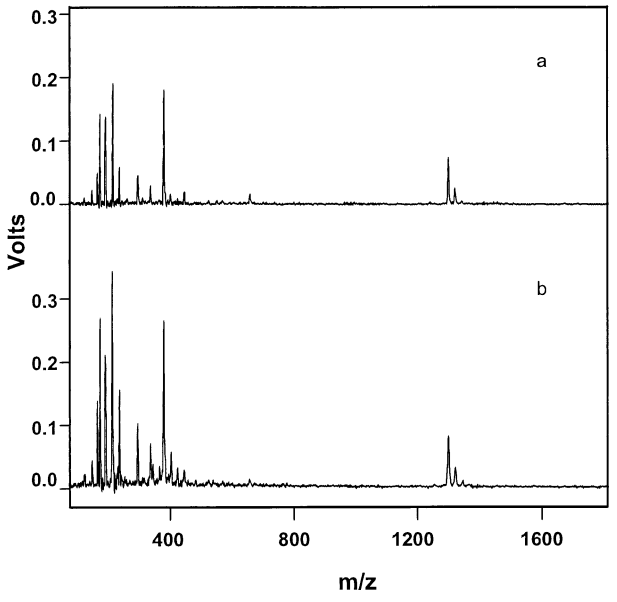

Figure 1. Representative MALDI mass spectra of angiotensin I applied to (**a**) PET and (**b**) PVDF polymer surfaces.

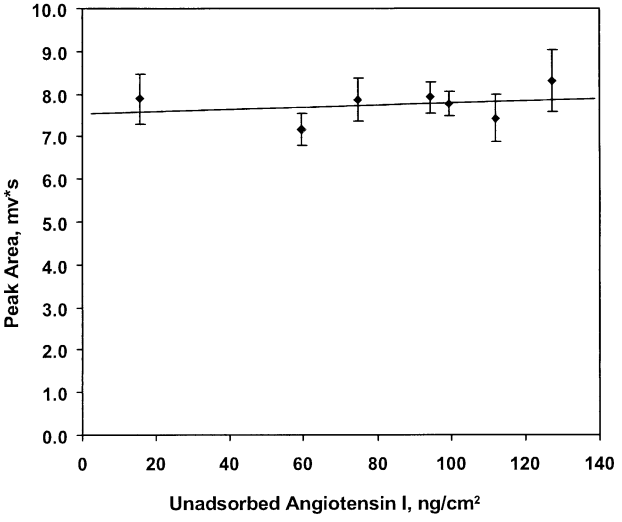

Figure 2. Plot of the difference between the applied and adsorbed angiotensin I (unadsorbed angiotensin I) vs. the integrated angiotensin I MALDI ion signal for angiotensin I applied to a variety of polymer substrates (surface concentration = 140 ng/cm^2).

were deposited on each of the polymer surfaces to yield surface concentrations of 140 ng/cm^2, based on the macroscopic surface area of the polymer disk. The ACHCA matrix and 10% TFA solutions were subsequently codeposited on the peptide coated polymer surfaces as previously described. Finally, the samples were introduced to the time-of-flight mass spectrometer and MALDI mass spectra were collected. Representative MALDI mass spectra of angiotensin I applied to PET and PVDF polymer surfaces are shown in Figure 1.

To evaluate the data obtained in this experiment, two correlations were examined. In the first analysis of the data the difference between the applied and adsorbed angiotensin I (the unadsorbed angiotensin I) was plotted versus the peptide MALDI ion signal (see Figure 2). The linear regression analysis of this plot yielded an R^2 value of 0.07, suggesting that no significant correlation exists between the reduction in angiotensin I MALDI ion signal and the surface-adsorbed peptide. In the second analysis the difference between the applied and retained angiotensin I (the unretained angiotensin I) was plotted versus the peptide MALDI ion signal (data not shown). The linear regression analysis of this plot yielded a significantly higher R^2 value of 0.50. However, a significant uncertainty in the evaluation of this data arises because the angiotensin I quantity applied generally exceeds the peptide quantity retained by the polymer surface by more than an order of magnitude. Thus, the impact of the surface-retained angiotensin I on the peptide MALDI ion signal would be expected to be relatively small.

To address the deficiency in the evaluation of the correlation between reductions in peptide MALDI signals and retention of the peptide by the polymer surface, a second group of experiments was performed. In this group of experiments the quantity of angiotensin I applied to each polymer surface was held constant at a value chosen to exceed by approximately 20% the quantity retained by the polymer with the highest retention affinity (PEEK). Similar to the approach previously described, the stock 1.0 mg/mL solution of angiotensin I was diluted to 0.0020 mg/mL and 2 μL aliquots of this solution were deposited on each of the polymer surfaces to yield surface concentrations of 22 ng/cm^2. The ACHCA matrix and 10% TFA solutions were subsequently codeposited on the peptide coated polymer surfaces as previously described and MALDI mass spectra were collected.

The data from this second group of experiments was evaluated by examining the correlation between the unretained angiotensin I and the peptide MALDI ion signal. For this data set an R^2 value of 0.95 was obtained and good linearity in the plot of unretained angiotensin I versus peptide MALDI ion signal was observed (see Figure 3). These results suggest that the angiotensin I which is retained by the polymer surface is not effectively ionized by the MALDI process. Thus, the peptide MALDI ion signals are reduced in intensity in proportion to the quantity of peptide which is strongly bound to the polymer surface.

Quantitation of Polymer Surface–Peptide Retention Affinity by MALDI Mass Spectrometry

A further important insight derived from the results shown in Figure 3 is that the intercept of the linear regression is close to the origin of the plot. This observation suggests that, as the quantity of peptide applied to a given polymer surface approaches the peptide retention affinity of the surface, the peptide MALDI ion

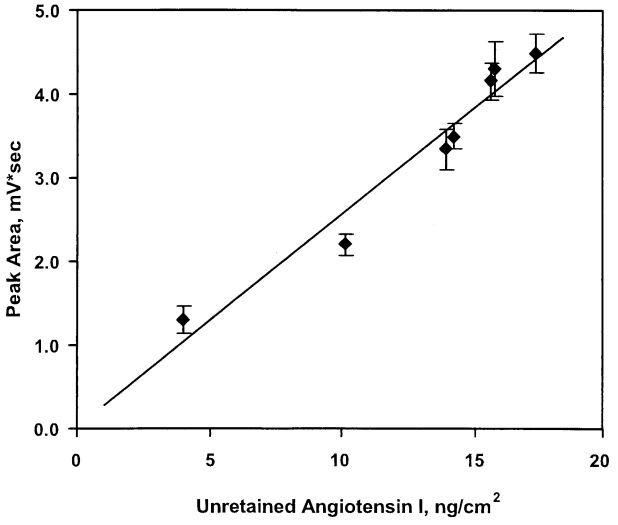

Figure 3. Plot of the difference between the applied and retained angiotensin I (unretained angiotensin I) vs. the integrated angiotensin I MALDI ion signal for angiotensin I applied to a variety of polymer substrates (surface concentration = 22 ng/cm^2).

signal approaches zero. Consequently, it should be possible to quantitate the surface–peptide retention affinity of a given polymer surface using a method of standard additions. Specifically, for a given polymer, a plot of quantity of peptide applied per unit area versus peptide MALDI ion signal should yield a nonzero x intercept which would correspond to the polymer surface–peptide retention affinity.

Figure 4 shows the results of a standard additions experiment performed using the PVDF polymer. In this

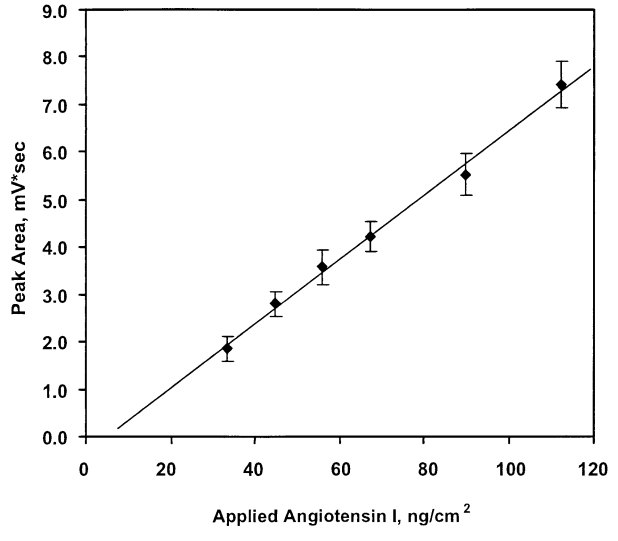

Figure 4. MALDI standard additions plot for angiotensin I applied to PVDF. Each data point represents the average integrated singly protonated angiotensin I ion signal and the error bars represent one standard deviation of the data set. A linear response is observed (R^2 = 0.995) with an x intercept, corresponding to the quantity of retained peptide, of 5.4 ± 2.4 ng/cm^2.

experiment angiotensin I solutions of various concentrations were made by dilution of the stock 1.0 mg/mL solution of the peptide with PBS. Deposition of 2.0 μL aliquots of the resultant solutions on 0.18 cm^2 PVDF disks lead to the surface concentrations shown in Figure 4. Subsequent preparation of the sample and acquisition of the angiotensin I MALDI mass spectra proceeded in the fashion previously described. From the plot of the amount of angiotensin I per unit area versus the integrated angiotensin I MALDI ion signal in Figure 4, it is immediately clear that a nonzero x intercept is obtained, i.e., the MALDI ion signal goes to zero at a finite amount of applied peptide. Based on the results presented in the previous section this value should correspond to the PVDF surface–angiotensin I retention affinity.

Table 1 compiles the observed surface–angiotensin I retention affinities (x intercepts) of the standard additions plots for angiotensin I applied to each of the various polymer surfaces. Also shown in Table 1 are the standard deviations of the x intercepts and the R^2 values. In all cases a minimum of six angiotensin I surface concentrations were included in the data set. From the values presented in Table 1 it is immediately clear that all of the standard additions plots have nonzero x intercepts. Furthermore, a comparison of the surface–angiotensin I retention affinities obtained from the x intercepts of the MALDI standard additions plots with the surface–angiotensin I retention affinities determined by using [^{125}I]angiotensin I shows that in all cases the values obtained using the two methods are in excellent agreement. In addition, it should be noted that the relative uncertainties in the surface–peptide retention affinities determined using the radiolabeled peptide binding and MALDI methods are similar in magnitude. Clearly these results suggest that the MALDI standard additions method can be used to efficiently determine the surface–peptide retention affinities for a wide variety of polymer materials.

Further confirmation of the effectiveness of the MALDI standard additions method for surface–peptide retention affinity determination has been obtained in studies using the peptide porcine insulin. Table 2 presents the surface–porcine insulin adsorption and retention properties of PVDF and PET as determined by the exposure of these surfaces to [^{125}I]porcine insulin. These studies were performed using the flow through cell and methods identical to those previously described for angiotensin I. Table 2 also gives the number of porcine insulin molecules adsorbed or retained per macroscopic unit area and the percent surface coverage. As stated previously for angiotensin I, these values should be viewed as upper limit coverages because the microscopic surface area may be larger than the macroscopic surface area. In addition, Table 2 gives the surface–porcine insulin retention affinities for PVDF and PET determined from the x intercepts of MALDI standard additions plots (representative MALDI mass spectra shown in Figure 5). A comparison of the surface–

Table 2. Adsorption / retention of [^{125}I]porcine insulin to polymer substrates and the *x* intercepts of the MALDI standard additions plots

	Adsorbed [^{125}I]angiotensin I[a]			Retained [^{125}I]angiotensin I[a]			MALDI *x* intercept[c]	
Polymer	ng/cm^2	molecules/cm^2	Coverage[b]	ng/cm^2	molecules/cm^2	Coverage[b]	ng/cm^2	R^2
PVDF	150 ± 70	1.6×10^{13}	69%	21 ± 9	2.2×10^{12}	9.6%	27 ± 7	0.986
PET	130 ± 40	1.4×10^{13}	60%	31 ± 11	3.2×10^{12}	14%	33 ± 7	0.967

[a]Polymer surface areas are calculated from macroscopic dimensions and do not account for surface microscopic morphological structure. The values shown are the average quantity of peptide present on three samples. The error corresponds to one standard deviation of the data set.
[b]Percent coverage calculated using a cross sectional area reported for porcine insulin (4.4×10^{-14} cm^2) in [10].
[c]A minimum of six surface concentrations were used to generate the standard additions plot. At each surface concentration a total of 20 MALDI mass spectra were acquired randomly from three samples. The error in the *x* intercept was calculated according to [17].

porcine insulin retention values given in Table 2 reveals that the values obtained using the radiolabeled peptide binding and MALDI standard additions methods are in excellent agreement.

The results presented clearly demonstrate that a correlation exists between the quantity of peptide which interacts strongly with a given polymer surface and the subsequent reduction in the peptide MALDI ion signal. The inefficient ionization of the surface retained peptides could reflect poor incorporation of the peptides into the MALDI matrix crystals [11–14] and/or, as recently suggested, poor intimate contact between the peptide and matrix crystals [15]. Regardless, this observation is consistent with previous conclusions drawn by Schreiner et al. in the MALDI analysis of peptides separated by gel electrophoresis and electroblotted onto polymer membranes [16].

The quantity of peptide inefficiently ionized appears to be most closely associated with the quantity of peptide which is retained by the polymer surface rather than the quantity which is simply adsorbed to the surface. This result is perhaps not surprising given that the retained peptide, as determined in these studies, interacts with the polymer surface with sufficient strength to resist removal upon washing of the surface with the surfactant SDS. However, the ability to derive similar quantitative measures of the surface–peptide retention affinity using the radiolabeled peptide binding and MALDI standard additions methodologies does allow a somewhat less obvious conclusion to be drawn. The results suggest that similar quantities of peptide are retained by the polymer surface under the very different solution chemical conditions of washing by SDS and exposure to the acidic matrix/TFA solutions.

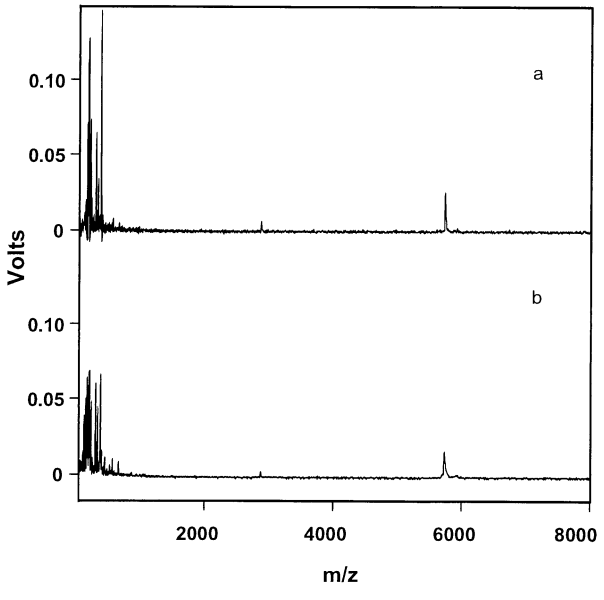

Figure 5. Representative MALDI mass spectra of porcine insulin applied to (**a**) PET and (**b**) PVDF polymer surfaces.

It should be pointed out, however, that the good agreement in the surface–peptide retention values determined by using the two methods may reflect specific properties of the peptides and polymer surfaces chosen for this initial study. In recent studies using angiotensin I and allylamine modified surfaces we have observed that the MALDI standard additions method yields lower surface–peptide retention affinities than ^{125}I-radiolabeled peptide binding studies. These results suggest that peptides are removed from these basic surfaces to a greater extent by the acidic matrix/TFA solution than by the SDS wash. This interpretation is indirectly supported by the observation that elimination of the TFA in the sample preparation step allows the surface–peptide retention affinities determined by the two methodologies to be reconciled.

In contrast to the amine modified surfaces, none of the polymers used in the present studies have pronounced acidic or basic character. Thus, the polymer surface–peptide interactions are likely primarily hydrophobic and/or hydrophilic in nature, rather than electrostatic. The agreement in the surface–peptide retention affinities determined by the radiolabeled peptide binding and MALDI standard additions methods suggests that these types of surface–peptide interactions can be disrupted to a similar extent by the surfactant and acidic matrix solutions.

Conclusion

Two important conclusions can be drawn from the current studies. First, the data presented clearly indicate

that strongly surface–retained peptides are not efficiently ionized under conventional MALDI conditions. This result has significant implications for research activities directed towards lowered MALDI limits of detection and/or quantitation by MALDI, particularly if surfaces with high retention affinities are employed. Second, it appears that a MALDI method of standard additions can be used to quantitate surface–peptide retention affinities under the specific chemical conditions experienced during the MALDI sample preparation process. More importantly, for hydrophobic/hydrophilic surface–peptide interactions it appears that the surface–peptide retention affinities obtained by the MALDI standard additions method correlate well with values determined by conventional ^{125}I-radiolabeled peptide binding. Thus, with proper development, the MALDI standard additions method could offer an efficient alternative method for the determination of this important biomaterial characteristic parameter.

Acknowledgments

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References

1. Wahlgren, M.; Arnebrant, T. *Trends Biotechnol.* **1991,** *9*, 201–208.
2. Andrade, J. D. *Surface and Interfacial Aspects of Biomedical Polymers*; Plenum: New York, 1985; Vol. 2.
3. Horbett, T. A.; Brash, J. L. *Proteins at Interfaces II, Fundamentals and Applications*; ACS Symposium Series 602; American Chemical Society: Washington, DC, 1995.
4. Van Delden, C. J.; Bezemer, J. M.; Engbers, G. H. M.; Feijen, J. *J. Biomater. Sci. Polymer Ed.* **1996,** *8*, 251–268.
5. Schmitt, A.; Varoqui, R.; Uniyal, S.; Brash, J. L.; Pusineri, C. *J. Colloid Interface Sci.* **1983,** *92*, 25–34.
6. Sarkar, D.; Chattoraj, D. K. *J. Colloid Interface Sci.* **1996,** *178*, 606–613.
7. Lee, S. H.; Ruckenstein, E. *J. Colloid Interface Sci.* **1988,** *125*, 365–379.
8. Walker, A. K.; Wu, Y.; Timmons, R. B.; Kinsel, G. R.; Nelson, K. D. *Anal. Chem.* **1999,** *71*, 268–272.
9. Chen, K.; Walker, A. K.; Wu, Y.; Timmons, R. B.; Kinsel, G. R. *J. Mass Spectrom.*, in press.
10. Gao, J.; Shitesides, G. M. *Anal. Chem.* **1997,** *69*, 575–580.
11. Strupat, K.; Karas, M.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1991,** *111*, 89.
12. Beavis, R. C.; Bridson, J. N. *J. Phys. D: Appl. Phys.* **1993,** *26*, 442.
13. Xiang, F.; Beavis, R. C. *Org. Mass Spectrom.* **1993,** *28*, 1424.
14. Fournier, I.; Beavis, R. C.; Blais, J. C.; Tabet, J. C.; Bolbach, G. *Int. J. Mass Spectrom. Ion Processes* **1997,** *169/170*, 19.
15. Horneffer, V.; Dreisewerd, K.; Lüdemann, H.-C.; Hillenkamp, F.; Läge, M.; Strupat, K. *Int. J. Mass Spectrom.* **1999,** *185/186/187*, 859–870.
16. Schreiner, M.; Strupat, K.; Lottspeich, F.; Eckerskorn, C. *Electrophoresis* **1996,** *17*, 954–961.
17. Harris, D. C. *Quantitative Chemical Analysis, 5th ed.*; W. H. Freeman: New York, 1998; p 104.