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Covalent Immobilization of Peptides on Self-Assembled Monolayer Surfaces Using Soft-Landing of Mass-Selected Ions

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Covalent immobilization of peptides on solid supports plays an important role in biochemistry with applications ranging from characterization of molecular recognition events at the amino acid level and identification of biologically active motifs in proteins to development of novel biosensors and substrates for improved cell adhesion.[1] Self-assembled monolayers (SAMs) provide a simple and convenient platform for tailoring chemical properties of surfaces.[2] Existing techniques for linking peptides to SAMs are based on solution-phase synthetic strategies and require relatively large quantities of purified material.[3] Here, we report a novel approach for highly selective covalent binding of peptides to SAMs using soft-landing (SL) of mass-selected ions. SL is defined as intact deposition of ions onto suitable substrates at hyperthermal (<100 eV) energies.[4,5] Recent studies have demonstrated that SAMs are excellent deposition targets for SL owing to their ability to dissipate kinetic energies of the projectiles and their efficiency in trapping captured species.[4,6,7] It has been proposed that SL could be utilized for controlled preparation of protein arrays.[5]

This study is the first account of covalent immobilization of mass-selected peptides on SAM surfaces using SL. We demonstrate efficient reactive landing (RL)[8−10] of several model peptides onto the SAM of *N*-hydroxysuccinimidyl ester terminated alkylthiol on gold (NHS-SAM). This method introduces exceptional selectivity and specificity into the surface preparation step by eliminating the effect of solvent and sample contamination on the quality of the film. In addition, the ability to focus and direct an ion beam at selected spots on the surface with variable sizes allows controllable fabrication of peptide patterns.

RGD sequence is by far the most effective and most frequently employed peptide motif for stimulated cell adhesion on synthetic surfaces.[11] It mediates cell adhesion by binding cellular integrin receptors. Covalent linking of RGD peptides to the substrate is essential for strong cell adhesion. Current strategies for preparation of such surfaces have been recently reviewed.[12] In this study we used cyclic pentapeptide c(-RGDfK-) (f = D-phenylalanine) as a model system.

NHS-SAM reacts with accessible primary amino groups in proteins or peptides by forming amide bonds. In the case of c(-RGDfK-), this reaction most likely involves the ϵ-amino group of the lysine side chain.[13] The experiment utilized the newly designed and constructed SL apparatus described elsewhere.[14] Briefly, doubly protonated, $[M + 2H]^{2+}$, ions of c(-RGDfK-) (m/z = 302.7) produced in an electrospray ionization (ESI) source are introduced into the vacuum system using an electrodynamic ion funnel, efficiently focused in a collisional quadrupole, mass selected using a quadrupole mass filter, and transferred to the surface through an electrostatic bending quadrupole and a series of einzel lenses. The optimized current of mass-selected ions delivered to the surface is 40 pA corresponding to deposition of ca. 1 ng of c(-RGDfK-) onto a 2 mm spot in 2 h (additional experimental details are described in the Supporting Information).

SAM surfaces before and after SL are characterized ex situ using 15 keV Ga^+ time-of-flight secondary ion mass spectrometry (TOF-SIMS) and grazing-incidence infrared reflection−absorption spectroscopy (IRRAS). Because IRRAS measurements require a larger amount of material, the corresponding samples were prepared by placing the SL target after the quadrupole mass filter that allowed us to deliver a much higher ion current of 400 pA to the surface and deposit ca. 18 ng of peptide onto a 5 mm spot in 4 h. Surfaces prepared by SL were analyzed before and after rinsing in methanol. For comparison, solution-phase covalent linking of c(-RGDfK-) to the NHS-SAM surface was performed by immersing the surface in a 2 mM solution of c(-RGDfK-) in the NH_4HCO_3 buffer (pH = 8) for 2 h followed by sonication in methanol for 5 min and thorough rinsing of the surface. Our systematic studies of the kinetics of c(-RGDfK-) attachment to the NHS-SAM surface in solution demonstrated that under these experimental conditions the maximum coverage (defined as 1 monolayer coverage) was achieved in about 3 h, while ca. 60% of the maximum coverage was obtained in 2 h.

Figure 1b shows TOF-SIMS spectra of the NHS-SAM surface following SL of 1 ng of mass-selected c(-RGDfK-). In addition to common surface-related peaks (Figure 1a), TOF-SIMS spectra obtained following SL contain numerous peptide-related peaks. Consistent with our previous studies,[7,15] the spectrum obtained following SL is dominated by the singly protonated, $[M + H]^+$, ion (m/z 604.3). Other major features include $[M + Au]^+$ (m/z 800.2) and peptide−SAM complexes at m/z 851.4 and 883.4. These species are almost completely removed from the surface by sonication and thorough rinsing in methanol (Figure 1c) suggesting that they are loosely bound to the NHS-SAM surface.

Interestingly, a significant number of peptide-related peaks are not affected by the rinsing procedure. Moreover, striking similarities between the spectrum shown in Figure 1c and the spectrum obtained for peptide grafted onto the NHS-SAM from solution (Figure 1d) suggest that these features correspond to peptide molecules covalently linked to the NHS-SAM surface. Specifically, the doublet observed at m/z 804.4 and 802.4 corresponds to the protonated covalent adduct (CA) between the peptide (603.3) and the thiol (315.2) without the NHS endgroup (115.1), $[CA + H]^+$, and its analogue that has an additional double bond. This doublet is an important signature of the covalent binding through formation of the amide bond. The covalent adduct is also observed as ion cationized on gold (m/z 1000.4), $[CA + Au]^+$. In addition, a series of fragment ions produced by high-energy bombardment of the surface in SIMS is observed in the TOF-SIMS spectrum. These include peaks corresponding to the loss of H_2S/S and up to 10 CH_2 groups from the doublet and $[CA + Au]^+$. The lower-mass part of the spectrum contains peaks corresponding to loss of the NHCO moiety from the amide bond between c(-RGDfK-) and the SAM chain and up to four CH_2 groups from the side chain of the lysine residue. Detailed labeling of these signature peaks is given in the Supporting Information. The observed fragmentation pattern provides strong support for covalent binding of c(-RGDfK-) to the NHS-SAM surface through the lysine side chain. Other lysine-

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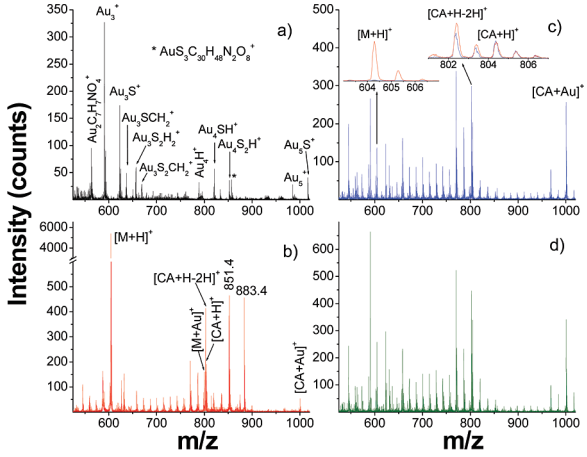

Figure 1. TOF-SIMS spectra of unmodified NHS-SAM (a); NHS-SAM with 1 ng soft-landed c(-RGDfK-) before (b) and after (c) rinsing; and NHS-SAM following 2 h solution-phase reaction (d).

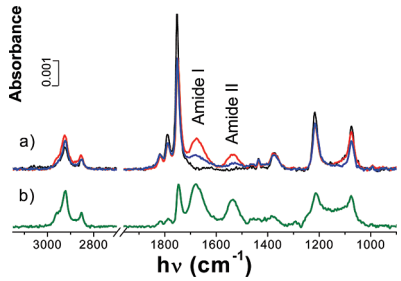

Figure 2. IRRAS spectra of unmodified NHS-SAM (a, black); NHS-SAM with 18 ng soft-landed c(-RGDfK-) before (a, red) and after (a, blue) rinsing; and NHS-SAM following 2 h solution-phase reaction (b, green).

containing peptides including GRGDSPK and KAAAA were also successfully linked to the NHS-SAM surface by RL.

Although TOF-SIMS is a very sensitive technique for molecules adsorbed on surfaces, secondary ion yields strongly depend on the substrate, coverage, and the binding energy between the adsorbate and the surface.[16] In this study we used IRRAS as a complementary surface characterization technique. Figure 2a compares IRRAS spectra obtained for the unmodified NHS-SAM (black), NHS-SAM following 4 h SL of ca. 18 ng of c(-RGDfK-) (red), and the same surface after extensive sonication and rinsing in methanol (blue), while Figure 2b shows the spectrum obtained for the NHS-SAM following 2 h solution-phase reaction.

Amide bands at 1676 and 1535 cm^{-1} are observed following SL of c(-RGDfK-) on the NHS-SAM surface (Figure 2a). Subsequent rinsing of this surface reduces the intensity of the amide bands by a factor of 2, indicating that about half of the observed IRRAS signal originates from peptide bonds of loosely bound c(-RGDfK) molecules. Covalent attachment of peptides to the surface also results in suppression of the bands characteristic of the NHS endgroup including the asymmetric stretch of the NHS carbonyls at 1751 cm^{-1}, the asymmetric CNC stretch of the NHS at 1217 cm^{-1}, and the NCO stretch of the NHS at 1074 cm^{-1}.

Peptide coverage on the surface can be estimated by comparing the spectrum shown in Figure 2a (blue) with the IRRAS spectrum obtained following surface modification using solution-phase chemistry (Figure 2b). Four times lower intensity of amide bands is observed for peptide deposited by RL (Figure 2a, blue) as compared with peptide grafted using solution-phase reaction (Figure 2b). It should be noted that while the latter produces SAM uniformly covered with peptide, RL deposits ions onto a smaller area on the surface. The diameter of the spot irradiated with ions of 5 mm was determined using the TOF-SIMS profile scan. Because IRRAS interrogates a 10 mm spot on the surface, the intensities of the bands observed in Figure 2b must be scaled down by a factor of 4 for comparison with the results obtained using RL. It follows that the local coverage of the peptide covalently linked using 4 h RL is comparable to the coverage of 60% of a monolayer obtained using 2 h reaction in solution.

In conclusion, we presented a novel approach for controlled immobilization of peptides on SAM surfaces using RL of mass-selected ions. Surface characterization using TOF-SIMS and IRRAS provides complementary information on the mode of binding and the amount of immobilized molecules on the surface. We demonstrated efficient covalent linkage of peptide ions to the NHS-SAM surface through the lysine side chain. It is remarkable that similar local surface coverage was achieved using 4 h RL and 2 h solution-phase reaction. Systematic studies focused on understanding the mechanisms and important factors that affect the efficiency of RL are underway in our laboratory. The results will be discussed in our forthcoming publications.

High selectivity and specificity inherent to mass spectrometry and ion beam chemistry provide unprecedented control for preparation of novel substrates for biorecognition studies and biomaterials for stimulated protein and cell adhesion.

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Supporting Information Available: Experimental procedures and TOF-SIMS spectrum of the NHS-SAM surface following SL with detailed peak assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

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