Preparation of α-Helical Peptide Arrays on Self-Assembled Monolayer Surfaces using Soft-Landing and Reactive Landing of Mass-Selected Ions**

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The α-helix – a common building block of the protein secondary structure – plays an important role in determining protein structure and function. The biological function of the α-helix is mainly attributed to its large macrodipole[1] originating from the alignment of individual dipole moments of peptide bonds. Preparation of directionally aligned α-helical peptide layers on substrates has attracted significant attention because the resulting strong net dipole is useful for a variety of applications in photonics,[2,3] molecular electronics,[4] and catalysis.[5-7] In addition, conformationally-selected α-helical peptide arrays can be used for detailed characterization of molecular recognition steps critical for protein folding, enzyme function and DNA binding by proteins. Existing technologies for the production of α-helical peptide surfaces are based on a variety of solution phase synthetic strategies[2,5,8-11] that usually require relatively large quantities of purified materials.

Preparative mass spectrometry based on soft-landing (SL)[12-24] of mass-selected ions is a viable alternative to the existing surface modification approaches. It has been demonstrated that SL enables highly specific preparation of uniform thin films of biological molecules on substrates.[25-28] In addition, reactive landing (RL), in which SL is followed by covalent linking of molecules to chemically reactive surfaces, can be used for controlled immobilization of peptides and proteins on solid supports.[29-31] Because SL is a relatively gentle ion deposition technique it is easy to preserve the primary structure of deposited species. However, it is very difficult to control the secondary structure of soft-landed biomolecules because electrospray ionization (ESI) utilized in these experiments generates ions in a variety of different conformations.

Here we present a first study focused on preparation of conformationally-selected peptide arrays using SL of mass selected peptide ions on self-assembled monolayer (SAM) surfaces. We selected the singly protonated Ac-A_{15}K peptide as a model system for this study because ion mobility measurements and molecular dynamics (MD) simulations demonstrated that this peptide forms a very stable α-helical conformation in the gas phase stabilized by the interaction between the protonated C-terminal lysine residue and the dipole of the helix.[32-35] We demonstrate formation of the α-helical peptide array on an inert SAM of alkylthiol on gold (HSAM) and covalent immobilization of the Ac-A_{15}K peptide on a reactive SAM of N-hydroxysuccinimidyl ester terminated alkylthiol on gold (NHS-SAM) with retention of the secondary structure. Because the NHS-SAM surface readily reacts with primary amino groups in proteins or peptides by forming amide bonds[36,37] this substrate has been previously used for efficient covalent immobilization of soft-landed peptides onto SAMs via the formation of an amide bond between the SAM and the amino group of the lysine side chain.[30,31]

Experiments were performed using an ion deposition apparatus described in detail elsewhere.[27] The instrument is equipped with a high-transmission electrospray ionization (ESI) source and a quadrupole mass filter. Singly protonated peptide molecules, [Ac-A_{15}K+H]$^+$ (m/z = 1254.6), produced by ESI were mass-selected and deposited onto SAM surfaces at the collision energy of 20 eV. Inert SAMs of dodecanethiol (HSAM) and reactive NHS-SAMs on gold were used as SL targets. The deposition time was controlled by monitoring the ion current on the surface. Typical SL experiments reported in this study correspond to deposition of ca. one monolayer of the peptide onto the surface. ESI deposition was performed by placing the target in front of the ESI emitter tip for controlled time duration. Secondary structure of the peptides in the electrospray solution (76μM Ac-A_{15}K in 50:50 (v/v) methanol/water with 1% acetic acid) was determined using circular dichroism (CD) spectroscopy. Infrared reflection absorption spectroscopy (IRRAS) and time of flight-secondary ion mass spectrometry (TOF-SIMS) were used to obtain structural information for soft-landed species.

IRRAS is used for characterization of the secondary structure of peptides on SAM surfaces based on the presence and position of amide I, amide II and amide A bands originating from peptide bonds.[11,38] The amide I band, commonly used for characterization of the peptide secondary structure using FTIR, is dominated by the C=O stretching vibrations of amide groups and gives rise to infrared absorption in the region between 1600 and 1700 cm^{-1}. The amide II band represents mainly N-H bending (60%) with some C-N stretching (40%) and usually appears at ~ 1550 cm^{-1}. Although fairly abundant, the amide II band is not very sensitive to changes in the peptide secondary structure. The amide A band responsible for absorption in the 3200-3300 cm^{-1} region corresponds to the stretching mode of the N-H bond. Because the N-H stretching vibration is strongly affected by the hydrogen bonding environment, this band is also sensitive to changes in the secondary structure.

Figure 1 compares standard CD spectra of the α-helix, β-sheet and random coil with the spectrum obtained for the ESI solution of Ac-A_{15}K. The CD spectrum of the Ac-A_{15}K solution shows the presence of a mixture of conformations dominated by the β-sheet and a small fraction of the α-helix and random coil. Similarly, the IRRAS spectrum of the Ac-A_{15}K layer prepared on the HSAM surface using ESI deposition is also dominated by the characteristic features of the β-sheet structure. Specifically, the IRRAS spectrum

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(blue trace, Figure 2) shows a broad amide I band with dominant features at 1632 and 1697 cm^{-1} characteristic of the β-sheet conformation.[39] In addition, the position of the amide A band centered at 3280 cm^{-1} indicates that NH groups are involved in C=O···H-N hydrogen bonds typical for the β-sheet structure. The features in the center of the amide I band (~1670 cm^{-1}) and the high-frequency tailing of the amide A band can be assigned to contributions of the α-helix and the random coil conformations.

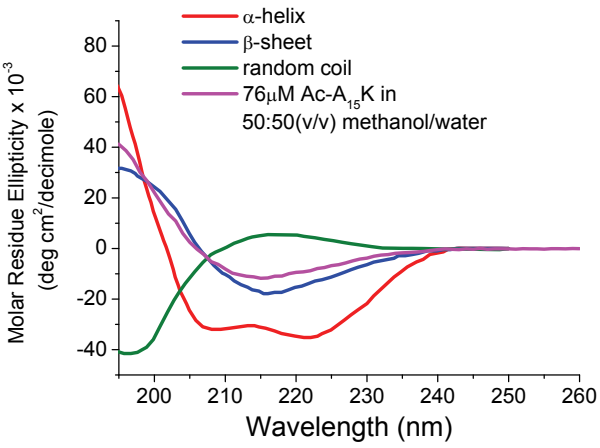

Figure 1. CD spectrum of the ESI solution of Ac-A_{15}K (76μM Ac-A_{15}K in 50:50 (v/v) methanol/water with 1% acetic acid) in comparison with standard CD spectra of pure α-helix, β-sheet and random coil conformation.

In contrast, the peptide layer prepared by SL of the singly protonated Ac-A_{15}K reveals significantly different IRRAS features (red trace, Figure 2). The spectrum is characterized by much narrower amide bands suggesting that there is only one major conformer. Furthermore, the positions of the amide I band at 1666 cm^{-1} and the amide A at 3307 cm^{-1} indicate that soft-landed Ac-A_{15}K exists in a nearly pure α-helical conformation.[39] Additional support for this assertion is provided by the presence and position of the peak at 1310 cm^{-1} in the amide III region (1200-1400 cm^{-1}). The amide III region has been rarely used for peptide characterization because of the low intensity of this band. However, recent studies demonstrated that the amide III band can be much more useful than the amide I band for the determination of the secondary structure of proteins or peptides because different conformations are better resolved in the amide III region.[40] For example, serious overlap of the random coil and the α-helix bands (<10 cm^{-1} difference) in the amide I region makes it difficult to distinguish between these conformations. In contrast, distinct differences in the position of the amide III band of the α-helix (1300 cm^{-1}) and the random coil (1240-1260 cm^{-1}) enables more accurate assessment of these secondary structure motifs.

IRRAS spectra shown in Figure 2 unambiguously demonstrate that SL of $[Ac\text{-}A_{15}K+H]^+$ onto the HSAM surface results in formation of a nearly pure α-helical peptide layer, while the β-sheet

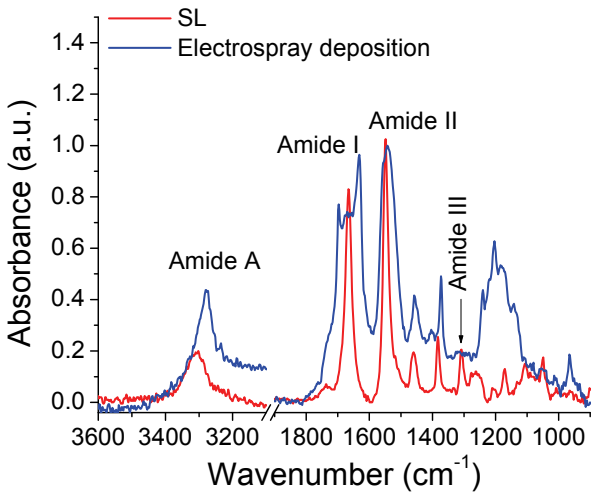

Figure 2. IRRAS spectra of the Ac-A_{15}K layer on the HSAM surface prepared by electrospray deposition (blue) and soft-landing (red). The HSAM background has been subtracted from both spectra.

structure dominates the layer prepared directly from solution. It is interesting to note that the α-helical peptide array prepared by SL of $[Ac\text{-}A_{15}K+H]^+$ onto the HSAM surface remained substantially unchanged at ambient environment for at least 20 days. However, the characteristic peptide bands are completely removed following extensive rinsing of the surface in trifluoroethanol (TFE) and methanol suggesting that soft landed Ac-A_{15}K molecules are loosely bound to the HSAM surface. It is generally accepted that solution-phase structures of biological molecules are not necessarily preserved in the gas phase. As mentioned earlier, ion mobility experiments demonstrated that gas-phase $[Ac\text{-}A_{15}K+H]^+$ ion adopts an extremely stable α-helical conformation. Our results suggest that the stable α-helical gas-phase conformation of the Ac-A_{15}K peptide molecule is successfully immobilized and preserved on the HSAM surface using ion soft-landing.

Covalent immobilization of the Ac-A_{15}K peptide was performed using the NHS-SAM surface as a SL target. Our previous studies showed that SL of lysine-containing peptides onto the NHS-SAM surface results in efficient formation of an amide bond between the peptide and the surface.[30,31] Figure 3 shows IRRAS spectra obtained for the unmodified NHS-SAM (black), NHS-SAM following SL of ca. one monolayer of $[Ac\text{-}A_{15}K+H]^+$ (red), the same surface after extensive rinsing in TFE and methanol (blue) and after subsequent immersion in TFE for three days (green). The IRRAS spectrum of the blank NHS-SAM surface is characterized by several NHS-related bands, 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}. Characteristic narrow amide bands at 3310 cm^{-1} (amide A), 1664 cm^{-1} (amide I), 1550 cm^{-1} (amide II) and 1310 cm^{-1} (amide III) are observed following peptide SL indicating that the immobilized Ac-A_{15}K retains the α-helical conformation on the NHS-SAM surface. In contrast with the results obtained for the HSAM surface, the intensity of amide bands was not completely eliminated by rinsing

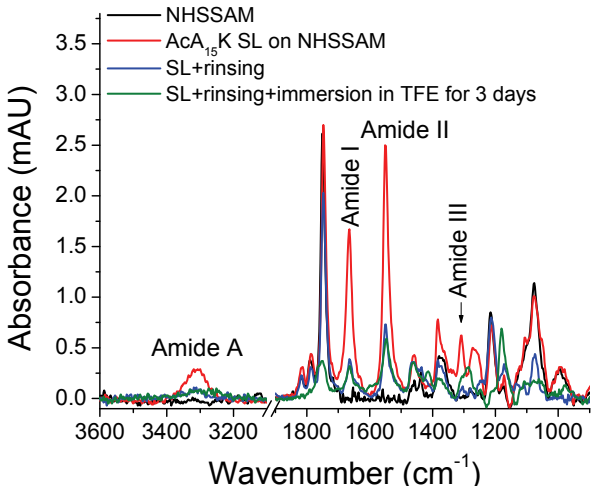

Figure 3. IRRAS spectra of unmodified NHS-SAM (black), NHS-SAM with ca. one monolayer of soft landed Ac-A_{15}K before (red) and after (blue) rinsing, and further immersion in trifluoroethanol (TFE) for 3 days (green).

of the NHS-SAM surface in TFE and methanol. The decrease in the intensity of the amide bands is attributed to the removal of loosely bound molecules from the surface by rinsing, while the remaining intensity corresponds to a fraction of Ac-A_{15}K that has been covalently immobilized on the NHS-SAM surface. In agreement with our previous studies[30,31] we observe that covalent binding of the peptide to the surface results in the loss of the NHS group reflected in the suppression of the bands characteristic of the NHS end group. The rinsed surface was further immersed in TFE for three days and subsequently characterized using IRRAS. Clearly, the amide band signal was not affected by this procedure while the NHS-related bands almost completely disappeared. Slow degradation of the NHS-SAM surface most likely involves transesterification reaction with TFE. In contrast, amide bonds are not reactive towards the solvent. It is remarkable that rinsing and immersion of the surface in TFE did not affect the shape and position of the amide bands indicating unprecedented stability of the α-helical conformation of Ac-A_{15}K covalently linked to the NHS-SAM surface.

Figure 4 compares TOF-SIMS spectra of the unmodified NHS-SAM and the NHS-SAM with ca. one monolayer of soft landed Ac-A_{15}K before and after rinsing in TFE and methanol. Consistent with our previous studies,[31,41] the SIMS spectrum obtained following SL is dominated by the singly protonated peptide molecule $[M+H]^+$ (m/z 1254.6). In addition, a series of abundant $\mathbf{b_n}$ (n=1-15) fragment ions of the peptide is observed in the spectrum. Minor products include $\mathbf{y_n}$, $\mathbf{y_n}$-NH_3 and several internal fragments. Similar fragment distribution (results not shown) was obtained using low-energy collision-induced dissociation (CID) suggesting that fragmentation observed in the SIMS spectrum results from the vibrational excitation of secondary ions. The signal of $[M+H]^+$ and some of the fragments almost completely disappears after sonication and thorough rinsing of the surface in TFE and methanol indicating that these species originate from loosely bound Ac-A_{15}K molecules. The remaining TOF-SIMS signal mainly contains the b-ion series that is not affected by the rinsing procedure. This signal is attributed to the fragments of Ac-A_{15}K molecules covalently bound to the NHS-SAM surface.

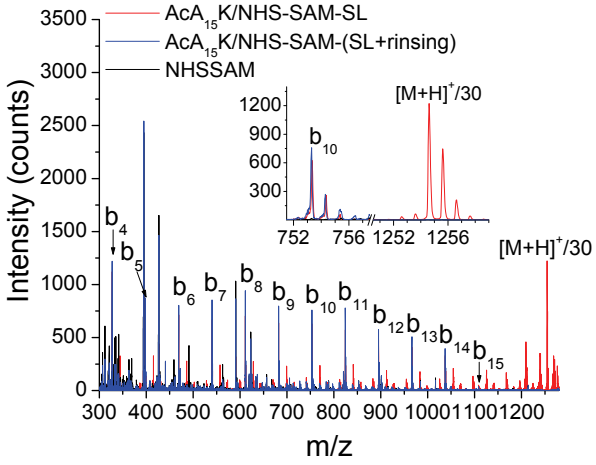

Figure 4. TOF-SIMS spectra of unmodified NHS-SAM (black), NHS-SAM with ca. one monolayer of soft landed Ac-A_{15}K before (red) and after (blue) rinsing.

It should be noted that abundant formation of b-ions for the singly protonated Ac-A_{15}K ion is rather unexpected because of the presence of a basic lysine residue at the C-terminus. Gas phase fragmentation of protonated peptides is typically dominated by cleavages of peptide (amide) bonds resulting in formation of **b** and **y** ions if the charge remains on the N-terminus or C-terminus, respectively.[42] The branching ratio of the N- and C-terminal product ions is largely determined by relative gas phase basicities of the corresponding neutral species.[43] It is generally accepted that CID spectra of peptides with C-terminal basic residues are dominated by the y-ion series.[42] However, both TOF-SIMS and CID spectra of Ac-A_{15}K show unexpectedly abundant b-ions. Similar fragmentation behavior was reported by van Oostrum and co-workers for $[M+H]^+$ ions of polyalanine-containing peptides with the C-terminal arginine residue.[44] Systematic studies performed by this group suggested that the enhanced b-ion formation is correlated with the α-helical structure of the fragmenting peptides. Abundant formation of b-ion series in the TOF-SIMS spectrum of soft landed Ac-A_{15}K on the NHS-SAM surface provides further support for the conclusion that these peptide molecules mainly adopt the α-helical conformation. Because covalently bound Ac-A_{15}K molecules are immobilized on the SAM surface through the amide bond between the amino group of the C-terminal lysine residue and the surface, formation of C-terminal y-ions from covalently linked molecules is very unlikely. As a result, b-ions are the only fragments observed in the TOF-SIMS spectrum after rinsing. It should be noted that the b-ion series is also dominant in the TOF-SIMS spectrum of Ac-A_{15}K loosely bound to the HSAM surface suggesting that these fragments

originate both from covalently and non-covalently immobilized α-helical Ac-A_{15}K molecules on the NHS-SAM surface.

In summary, we demonstrated for the first time that SL of mass-selected peptide ions can be used for preparation of conformation-specific peptide arrays on SAM surfaces. $[Ac\text{-}A_{15}K+H]^+$ used as a model system in this study adopts stable α-helical conformation in the gas phase. IRRAS and TOF-SIMS were used to obtain structural information of the soft landed peptide molecules. Our results showed that while the Ac-A_{15}K grafted onto SAM surfaces from solution favored the β-sheet structure, deposition of $[Ac\text{-}A_{15}K+H]^+$ ions from the gas phase resulted in formation of a stable α-helical peptide layer on SAM surfaces. Furthermore, we found that soft-landed peptide molecules retained the α-helical conformation at ambient environment for at least 20 days. Deposition of $[Ac\text{-}A_{15}K+H]^+$ onto the reactive NHS-SAM resulted in covalent immobilization of the α-helical conformation on the surface. It is remarkable that the covalently linked peptide molecules retained their gas-phase conformation following extensive rinsing and extended immersion in TFE. This study presents a first step towards controlled immobilization of conformationally pure peptides and proteins on solid supports using soft- and reactive landing. Our results suggest that the combination of the ion mobility separation with soft- and reactive-landing experiments could be used in future studies for conformation enrichment and preparation of conformation-specific peptide and protein arrays.

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Conformation-Specific Peptide Arrays

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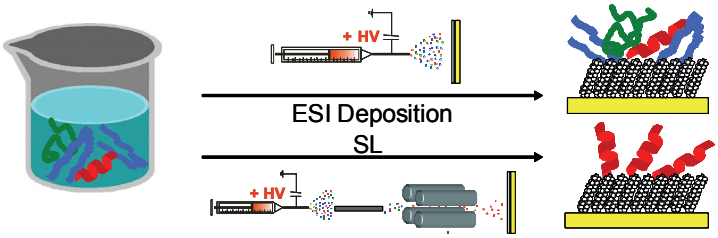

Soft-landing of $[Ac\text{-}A_{15}K+H]^+$ ions on self-assembled monolayer (SAM) surfaces is compared with deposition of a peptide layer directly from solution. The peptide used as a model system in this study adopts a stable α-helical conformation in the gas phase and a mixture of conformations dominated by the β-sheet in solution. Deposition of $Ac\text{-}A_{15}K$ from solution results in formation of a peptide layer dominated by the β-sheet structure, while a stable α-helical peptide layer on SAM surfaces is formed by soft-landing. This study shows that soft-landing of mass-selected peptide ions can be used for preparation of conformation-specific peptide arrays on SAM surfaces.