Peptide Arrays

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Helical Peptide Arrays on Self-Assembled Monolayer Surfaces through Soft 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] that usually require relatively large quantities of purified materials.

Preparative mass spectrometry based on soft landing (SL)[9–18] 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.[19–21] 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.[22,23] 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. Previous studies reported retention of the secondary and possibly tertiary structure by soft-landed proteins.[19a,21,22] Herein, we demonstrate that SL can be used to prepare peptides on substrates in stable conformations that do not exist in solution.

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This study focuses on the preparation of conformationally-selected peptide arrays using SL of mass selected peptide ions on self-assembled monolayer (SAM) surfaces. The singly protonated Ac-A_{15}K peptide was selected 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, which is stabilized by the interaction between the protonated C-terminal lysine residue and the dipole of the helix.[24] Formation of the α-helical peptide array is demonstrated 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[25,26] this substrate has been previously used for efficient covalent immobilization of soft-landed peptides onto SAMs by the formation of an amide bond between the SAM and the amino group of the lysine side chain.[23]

Experiments were performed using an ion deposition apparatus described in detail elsewhere.[20] Experimental details are given in the Supporting Information. Infrared reflection absorption spectroscopy (IRRAS) was used for characterization of the secondary structure of peptides on SAM surfaces based on the presence and position of amide I and amide A bands originating from peptide bonds.[11,27] The amide I band 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}, whereas the amide A band responsible for absorption in the 3200–3300 cm^{-1} region corresponds to the N–H stretching mode.

In Figure 1, standard circular dichroism (CD) spectra of the α helix, β sheet, and random coil are compared 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 (blue trace, Figure 2) shows a broad amide I band with dominant features at 1632 and 1697 cm^{-1} which is characteristic of the β-sheet conformation.[28] 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 (ca. 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

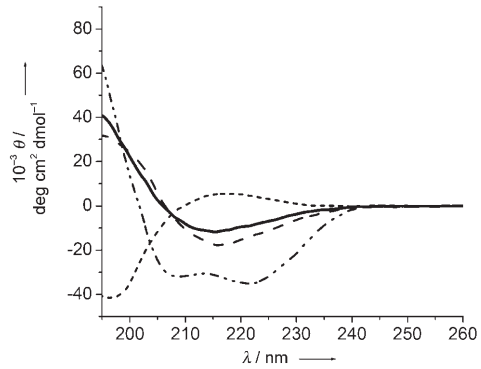

Figure 1. CD spectrum of an ESI solution of Ac-A_{15}K (76 μM) in 50:50 v/v methanol/water with 1% acetic acid (——) in comparison with standard CD spectra of pure α-helix (—·—·—), β-sheet (– – –) and random-coil (- - - -) conformations.

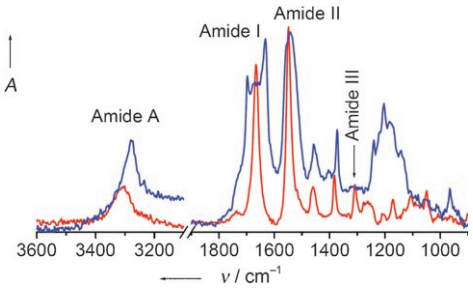

Figure 2. IRRAS spectra of the Ac-A_{15}K layer on the HSAM surface prepared by electrospray deposition (blue) and soft landing (red). The HSAM background has been subtracted from both spectra.

conformations. Similar results were obtained for a sample prepared by the dried-droplet approach.

In contrast, the peptide layer prepared by SL of singly protonated Ac-A_{15}K reveals significantly different IRRAS features (red trace, Figure 2). The spectrum has much narrower amide I and II bands, suggesting that there is only one major conformer. Because the β-sheet conformation yields a strong and sharp feature in the amide A region, the contribution from other conformations present in the ESI solution to this IR feature results in a relatively weak high-frequency shoulder. Furthermore, the positions of the amide I band at 1666 cm^{-1} and the amide A band at 3307 cm^{-1} indicate that soft-landed Ac-A_{15}K exists in a nearly pure α-helical conformation.[28] Additional support for this assertion is provided by the presence and position of the peak in the amide III region (1200–1400 cm^{-1}) at 1310 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.[29] For example, severe 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.

The IRRAS spectra given in Figure 2 demonstrate unambiguously that SL of $[Ac\text{-}A_{15}K + H]^+$ onto the HSAM surface results in formation of a nearly pure α-helical peptide layer, whereas the β-sheet 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 after exposure to laboratory air 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 above, 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 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. 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.[23] The IRRAS spectrum of the blank NHS-SAM surface (Figure 3) is characterized by several NHS-related bands, including the asymmetric stretch of the NHS carbonyl groups 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 of the NHS-SAM surface in TFE and

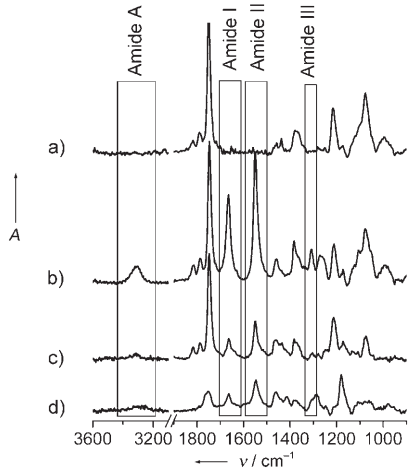

Figure 3. IRRAS spectra of a) unmodified NHS-SAM, NHS-SAM with ca. one monolayer of soft-landed Ac-A_{15}K before (b) and after (c) rinsing in TFE and methanol, and d) further immersion in trifluoroethanol (TFE) for 3 days.

methanol. The decrease in the intensity of the amide bands is attributed to the removal of loosely bound molecules from the surface by rinsing, whereas the remaining intensity corresponds to a fraction of Ac-A_{15}K that has been covalently immobilized on the NHS-SAM surface. Clearly, the amide band signal was not affected by this procedure whereas the NHS-related bands almost completely disappeared. Slow degradation of the NHS-SAM surface most likely involves a 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.

In summary, it has been 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. $[\text{Ac-A}_{15}\text{K}+\text{H}]^+$ used as a model system in this study adopts stable α-helical conformation in the gas phase. IRRAS was used to obtain structural information of the soft-landed peptide molecules. Our results show that although Ac-A_{15}K grafted onto SAM surfaces from solution favor the β-sheet structure, deposition of $[\text{Ac-A}_{15}\text{K}+\text{H}]^+$ ions from the gas phase results in formation of a stable α-helical peptide layer on SAM surfaces. It is reasonable to assume that if the conformational transition to the most stable α-helical structure of the gas-phase-protonated peptide is associated with a substantial free energy barrier, it is facilitated by collisions with the background gas in the high-pressure interface of our instrument. Furthermore, we found that soft-landed peptide molecules retained the α-helical conformation in the ambient environment for at least 20 days. Deposition of $[\text{Ac-A}_{15}\text{K}+\text{H}]^+$ 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 both soft landing and reactive landing. Our results suggest that the combination of the ion mobility separation with soft-landing 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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