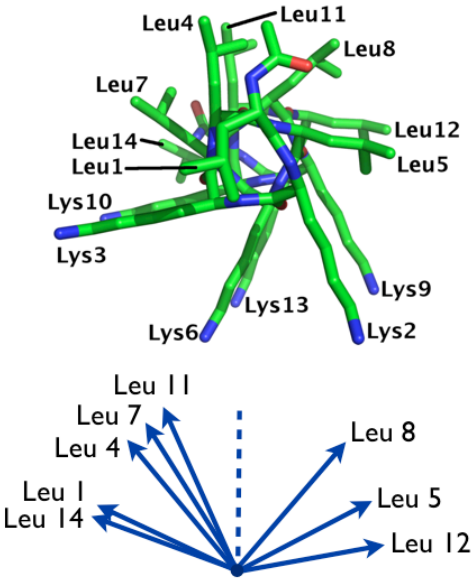
Side-Chain Specific Structural Studies of Peptides at Interfaces Using Isotope Labeling with SFG Spectroscopy

Tobias Weidner,[1] Nicholas F. Breen,[2] Gary P. Drobny,[2] David G. Castner[1]
[1]Departments of Chemical Engineering and Bioengineering, [2]Departments of Physics and Chemistry
University of Washington, Seattle WA, USA

Statement of Purpose: Controlled immobilization of peptides onto artificial biointerfaces plays a key role in antifouling, implant and immunosensor technologies. It is of crucial importance to develop tools to examine the structure of adsorbed peptides with molecular level resolution. Sum frequency generation (SFG) spectroscopy can probe proteins in situ at the solid-liquid interface and can provide information about the secondary structure and side chain order and orientation. Isotope labeling can address specific protein regions but its potential in conjunction with SFG spectroscopy has remained mostly unexplored. We combine these techniques to characterize the structure and the orientation of specific side chains of synthetic model peptides on surfaces in phosphate buffer saline (PBS) solutions. The analysis is supported with complemetary deuterium solid state nuclear magnetic resonance (NMR) experiments.

Methods: The peptide used is a 14-mer of hydrophilic lysine (K) and hydrophobic leucine (L) residues with an α-helical secondary structure. (DeGrado, WF. J Am Chem Soc. 1985;107:7684-89.) These LK14 peptides have the hydrophobic side chains on one side of the helix and the hydrophilic ones the other (Figure). Deuteration of the isopropyl group of each leucine residue (8 total samples) was used to probe their orientation when LK14 was adsorbed onto a hydrophobic polystyrene surface. In an additional experiment on charged surfaces we labeled the terminal NH_3 groups of all lysine side chains with ^{15}N to clarify the origin of the 3300 cm^{-1} NH feature observed in many protein SFG spectra. The SFG experiments were performed in situ in PBS in a liquid cell. The substrates for peptide adsorption were prepared on one side of an equilateral prism.

Results: Strong C-H stretches related to the leucine residues were observed for unlabeled LK14 on polystyrene. This is commonly associated with hydrophobic interactions of those side chains with the polystyrene surface.(Mermut, O. J Am Chem Soc. 2006; 128:3598-07) Phase sensitive measurements on coated gold substrates support this view and prove that the leucines are oriented towards the interface.(Weidner, T. Langmuir, in press.) Amide I SFG spectra confirm the peptides maintain their α-helical secondary structure on the surface. Site selective deuterium substitution at individual leucine side chains allowed us to obtain a more detailed picture of the structure of the adsorbed peptides. The tilt and torsion angles of the terminal isopropyl units of individual leucine side chains were determined by analyzing C-D stretching range SFG spectra between 2300 at 2000 cm^{-1}. We used the ratios of the symmetric and asymmetric C-D_3 stretching mode intensities acquired with different polarization combinations. The lower panel of the Figure summarizes pertinent isopropyl orientations.



We found that the orientation of the leucine side chains in the surface-bound LK14 was remarkably similar to the solution structure. The latter structure is shown in the upper panel of the Figure. Solid-state NMR data acquired on polystyrene beads is in line with the determined orientation change upon binding. Modeling the line shapes in of NMR spectra of LK14 adsorbed onto polystyrene beads with deuterated leucine incorporated at specific positions showed, that, in general, more inclined leucines assume a greater distance to the surface. (Breen, NF. J Am Chem Soc. 2009;131:14148-49.) In addition, ^{15}N labeling was used to address the controversial assignment of a pronounced peak near 3300 cm^{-1} observed for a variety of adsorbed proteins. This spectral feature has been assigned to both N-H containing side chains and backbone-related amide A resonances before. ^{15}N labeling of the lysine side chains resulted in a 9 cm^{-1} red-shift of this peak in the spectrum of LK adsorbed onto a SiO_2 surface, showing the 3300 cm^{-1} feature is related to the terminal amine group on the lysine side chains. (Weidner, T. J Phys Chem. C, in press.)

Conclusions: We have determined the orientations of all leucine side chains of LK14 on polystyrene using deuterium labeling strategies with SFG. The structure of the adsorbed peptide is strikingly similar to the calculated solution structure. This lets us conclude that not only the secondary structure, but also the orientation of the binding side chains remains relatively unaltered upon adsorption. On charged surfaces we have shown that the frequently observed 3300 cm^{-1} feature is related to lysine side chains binding the surface. Future experiments combining isotope labels and SFG will focus on more complex peptides and proteins.