Comparative Analysis of Fibronectin Using In Situ ToF-SIMS, SPI-MS, and dropDESI-MS in a Microfluidic Reactor

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Introduction

Fibronectin is a protein within the extracellular matrix of animal cells. It is an important biomolecule due to its role in cell differentiation, growth, kinesis and adhesion. Biological responses as such occur within aqueous environments and are mediated through membrane recognition and signaling; where fibronectin is found to play a role. Studying the outer molecular surface of fibronectin, a model system for proteins, in aqueous solution accurately represents fibronectin’s chemical components, made possible by the vacuum compatible microfluidic reactor.

Experimental Design

System for Analysis at the Liquid Vacuum Interface (SALVI), PDMS microfluidic block placed in vacuum chamber enabling in situ liquid MS. - Microfluidic channel - Aperture for direct liquid probing

Time-of-flight secondary ion mass spectrometer (ToF-SIMS) - Interface surface technique - Bismuth liquid metal ion beam - Monitors positive and negative emitted ions

Advanced light source single photon ionization mass spectrometer (ALS SPI-MS) - Interface surface technique - Synchrontron vacuum ultraviolet (VUV) photon beam - Determines appearance energy (AE)

Drop desorption electrospray ionization mass spectrometer (dropDESI-MS) - Ambient conditions - Electrode spray ion source - Capillarygenerates charged micro droplets

Results

ToF-SIMS

The m/z spectral plot for photoionization efficiencies (PIE) from 8.0 to 11.0 eV with a step size of 0.1 eV.

Table 2. Possible peak identification from the SPI-MS positive spectra.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>Cys-Val-Cys-Leu</td>
<td>tryptophan fragment</td>
</tr>
<tr>
<td>74</td>
<td>Cys-Val-Thr-Asp-Ser-Gly</td>
<td>glutamic acid</td>
</tr>
<tr>
<td>83</td>
<td>Cys-Val-Cys-Arg-Pro</td>
<td>methionine</td>
</tr>
<tr>
<td>131</td>
<td>Cys-Val-Cys-Leu</td>
<td>tyrosine</td>
</tr>
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Table 3. Summary of Estimated AE Values

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<th>m/z</th>
<th>AE Values</th>
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<tr>
<td>149</td>
<td>9.2 ± 0.1</td>
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<tr>
<td>83</td>
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Table 4. Possible peak identification from the drop DEISA-MS positive spectra.

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Discussion

Compounds and constituents of the fibronectin samples show complementary results based on the identified amino acid fragments. Possible m/z identifications for ALS SPI-MS and dropDESI-MS were calculated based on fibronectin’s amino acid sequence, while values for ToF-SIMS were comparable to previously conducted experiments. The microfluidic reactor successfully enabled different MS techniques in aqueous solution. Only fibronectin in aqueous solution has been studied so far in MS. Our results suggest the need for further research of large biomolecules to understand their surface compositions in aqueous solution, accurately representing their natural environment.

Acknowledgments

Rachel Komorek, Aala Al Hasan, LeVin Confin (administration), Yigang Fang and the TIF-SIMS research team at PNNL, LSBN, collaborators Masa Ahmed and Tyler Troy. Funding from NSF, M3D LDRD, TIC LDRD and DOE BER EMSL user facility.

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References

[8] Courtesy of LBNL

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