Results and Discussion

The biggest single problem with solution-phase H/D exchange as a mass spectrometric probe of surface exposure in a protein (or protein complex) is back-exchange of H for D after the initial H/D exchange has been quenched. Back-exchange results in loss of pertinent data and also greatly hampers data analysis. Previously, very fast, cold (0-4 °C) high-performance liquid chromatography (HPLC) was performed to help reduce back-exchange, but back-exchange calculation still averaged ~30%. Here, supercritical fluid chromatography replaces HPLC as the desalting/separation technique prior to mass analysis, providing a dramatic reduction in back-exchange compared to the fast, cold HPLC methods. The Figure shows electrospray ionization time-of-flight (ESI-TOF) mass spectra following SFC of the pentapeptide IFVQK. (Top) 10 μL injection at 4 pM/μL of the undeuterated peptide to demonstrate that the peptide was retained on the SFC column. (Bottom) Fully deuterated sample (loaded at the same concentration) that had been stored in deuterated buffer for several days. Back-exchange is only 7.5% based on the theoretical maximally exchanged mass for this pentapeptide with four exchangeable amide hydrogens.

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References