Isotope-Edited FTIR in H_2O: Determination of the Conformation of Specific Residues in Model α-Helix Peptide by ^{13}C Labeled Carbonyls

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Supplemental Information

Experimental Sections

Materials. Hydrochloride, trifluoroacetic acid, sodium chloride, and organic solvents (such as DMF, CH_2Cl_2, acetonitrile, and so on) were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA). All aqueous solutions were prepared using Millipore water (18 MΩ•cm). Piperidine was from Sigma Aldrich Co. (St. Louis, MO). Wang resin, diisopropylcarbodiimide (DIC) and 1-hydroxylbenzotriazole (HOBT), and Fmoc protected amino acids used for peptidolipid synthesis were purchased from AnaSpec Inc. (Fremont, CA). All the amino acids were in L-configuration.

Synthesis, Purification, and Mass Measurement of Pep17. Both unlabeled and ^{13}C labeled Pep17 were synthesized via solid phase (Fmoc) chemistry. DIC and HOBT were used for coupling reactions and 20% piperidine solution in DMF (v/v) was used for deprotection. After lyophilization, the crude product was purified by semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) on Waters Breeze 2 separation system equipped with 1525 EF binary pump and a column (Jupiter-10-C18-300, 10 mm i.d. × 250 mm) from Phenomenex (Torrance, CA). The mobile phases were 0.1% trifluoroacetic acid in water (v/v, mobile phase A) and 0.1% trifluoroacetic acid in acetonitrile (v/v, mobile phase B). The elution gradient was 10–16% B for 40 min at a flow rate of 4.7 mL/min. The Mass spectra of Pep17 were obtained from a Waters SYNAPT q-TOF tandem mass spectrometer, which was equipped with electron spray ionization and APCI ion source.

Circular Dichroism Measurement. The circular dichroism (CD) spectra were measured by a JASCO J-815 spectropolarimeter. Quartz cell of 1-mm and 0.2 mm path length were used to analyze Pep17 solution at pH 5.0 and 12.5, respectively. The spectrum was recorded with a response time of 4 s and a scan speed of 20 nm/min at 15 °C. The concentration of Pep17 samples was determined by the absorption of Tyr at 275 nm (extinction coefficient at 1.4 x 10^3 $M^{-1}cm^{-1}$) and at 293 nm (extinction coefficient at 2.4 x 10^3 $M^{-1}cm^{-1}$) under pH 5.0 and 12.5, respectively, using a UV-2600 spectrophotometer (Shimadzu Inc., Japan) with a 1 cm x 1 cm quartz cell.

*ATR FTIR Spectroscopy Measurement***.** The measurement of ATR FTIR spectra of either unlabeled or ^{13}C labeled Pep17 were performed on an EQUINOX 55 FTIR spectrometer (Bruker Optics, Billerica, MA) equipped with an BioATR-cell II unit accessory on the baseplate A729/q with a resolution of 2 cm^{-1} and co-addition of 128 scans. The IR beam was conducted out of the spectrometer and introduced into the BioATR-cell II accessory. The reflected IR beam was diverted to a HgCdTe (MCT) detector cooled by liquid nitrogen. The pure water and water solution of NaOH at pH 12.5 was used as the background for measuring spectra of Pep17 peptide at pH 5.0 and 12.5, respectively. The concentration of both unlabeled and labeled Pep17 was also determined by UV-Vis spectroscopy (shown in the CD measurements above). The concentration of Pep17 was in the range of 2.0 ~ 6.0 mg/ml.

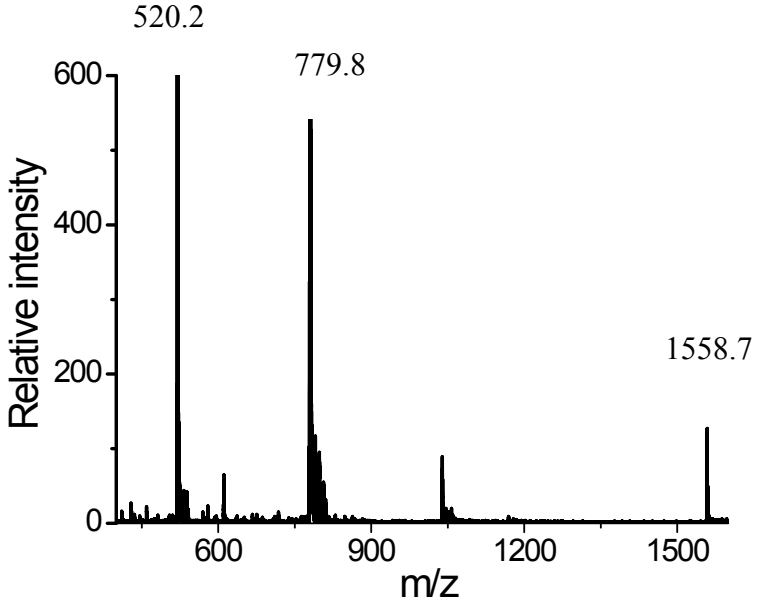

Figure S1. Mass spectra of L1 (double ^{13}C labeled Pep17 at residues 2 and 3)