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The ^{13}C Amide I Band is Still Sensitive to Conformation Change When the Regular Amide I Band Cannot Distinguish at the Typical Position in H_2O

Shanghao Li,[a] Joseph Dale Combs,[b] Olaa Eid Alharbi, [b] Jing Kong,[b] Chengshan Wang, * [b] and Roger M. Leblanc* [a]

[a] Department of Chemistry, University of Miami, Coral Gables, FL 33146

[b]Department of Chemistry, Middle Tennessee State University, Murfreesboro, TN 37132

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 Milli-Q 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 peptide synthesis were purchased from AnaSpec Inc. (Fremont, CA). All the amino acids were in L-configuration.

Synthesis and Purification of Pep25. Both unlabeled and ^{13}C labeled Pep25 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 cleavage of the peptide from the resin, the crude product was purified by semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) on a 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.

Mass Measurements. The Mass spectra of Pep25 were obtained from a Waters SYNAPT q-TOF tandem mass spectrometer operated in electron spray ionization. The sample cone temperature was 150 °C and the capillary voltage was 3000 V. A nitrogen flow rate of 500 L/h was used to disolvate the aerosols of the Pep25 aqueous solutions, which contains 0.1 % of trifluoroacetic acid. Positive ion mode was used for MS and MS/MS measurements, which used high purity of argon as collision gas at the collision energy of 3500 V. The peak of 1058.3 shown in Figure 1 was used as a precursor cation for fragment MS/MS experiment. To make it easier to distinguish the m/z peaks of various fragments of Pep25, the original MS/MS spectrum containing multiple charged fragments of Pep25 was converted (by the processing option "Maxent 3" in the software package of "Masslynx" in the computer of the Waters SYNAPT q-TOF tandem mass spectrometer) to a spectrum which contains only monoprotontated fragments as shown in Figure S2. The peaks in Figure S2 are easy to compare with the theoretical values as shown in Table S1.

Circular Dichroism Measurement. The circular dichroism (CD) spectra were measured by a JASCO J-810 spectropolarimeter. Quartz cell of 1-mm path length were used to analyze Pep25 solution in pure water under various conditions. The spectrum was recorded with a response time of 4 s and a scan speed of 20 nm/min at the corresponding temperatures. The concentration of Pep25 samples was determined by the absorption of Tyr at 275 nm (extinction coefficient at 1.4 x 10^3 $M^{-1}cm^{-1}$) under pH 6.0 by Shimadzu UV-2600 spectrometer.

*ATR FTIR Spectroscopy Measurement***.** The measurement of ATR FTIR spectra of either unlabeled or ^{13}C labeled Pep25 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 was used as the background for measuring spectra of Pep25 peptide. The concentration of both unlabeled and labeled Pep25 was also determined by UV-Vis spectroscopy (shown in the CD measurements above). The concentration of Pep25 was in the range of 5.7 ~ 12.2 mg/ml.

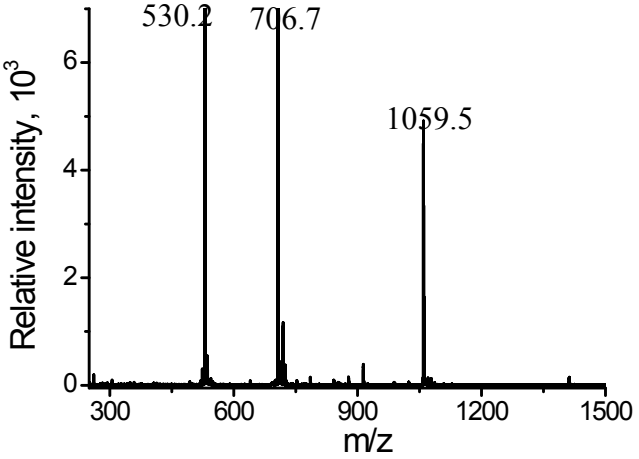

Figure S1. Mass spectra of L1 (double ^{13}C labeled Pep25 at residues 9 and 13).

Confirmation of the Pep25 Sequence by MS/MS. The fragment MS/MS spectrum of unlabeled Pep25 was shown in Figure S2. The molecular peak of unlabeled Pep25 detected at 2115.3 is close to the

theoretical value at 2115.7. Since each amide bond (O=C–N–H) in the backbone of Pep25 could be broken by the collision gas atom (i.e., argon in this paper), all the possible MS/MS fragment sequences are listed in Table S1. For example, when the C-terminal alanine was cleaved from Pep25 via the collision induced decomposition (CID), the sequence of AAAKAAAAKAAAAKAAAAKAAAAY (shown in the line 1 of Table S1) with the expected formula weight of 2026.4 was generated. This matched the fragment peak at 2026.3 detected in Figure S2. For the lost of three alanine fragment (i.e., AAA) via CID, the sequence of AKAAAAKAAAAKAAAAKAAAAY (theoretical formula weight of 1884.2 shown in the line 3 of Table S1) was generated together with the fragment of AAA simultaneously. The peak of AKAAAAKAAAAKAAAAKAAAAY was detected at 1883.9 and the peak of AAA was at 231.1. All of the N-terminal fragment sequences and their complementary C-terminal sequences are listed in Table S1 were detected in Figure S2. Thus, the sequence of unlabeled Pep25 can be confirmed. As for the labeled Pep25, similar method as mentioned above was used and all of the sequences listed in the paper were confirmed. Other peaks than those listed in Table S1 were also detected in Figure S2, because the MS/MS fragments were also generated when other bonds (not amide bond) were broken by collision gas. Due to the presence of many small background peaks below m/z value 200, these peaks are not useful for distinguishing Pep25 derived fragments and were not included in Figure S2 or Table S1.

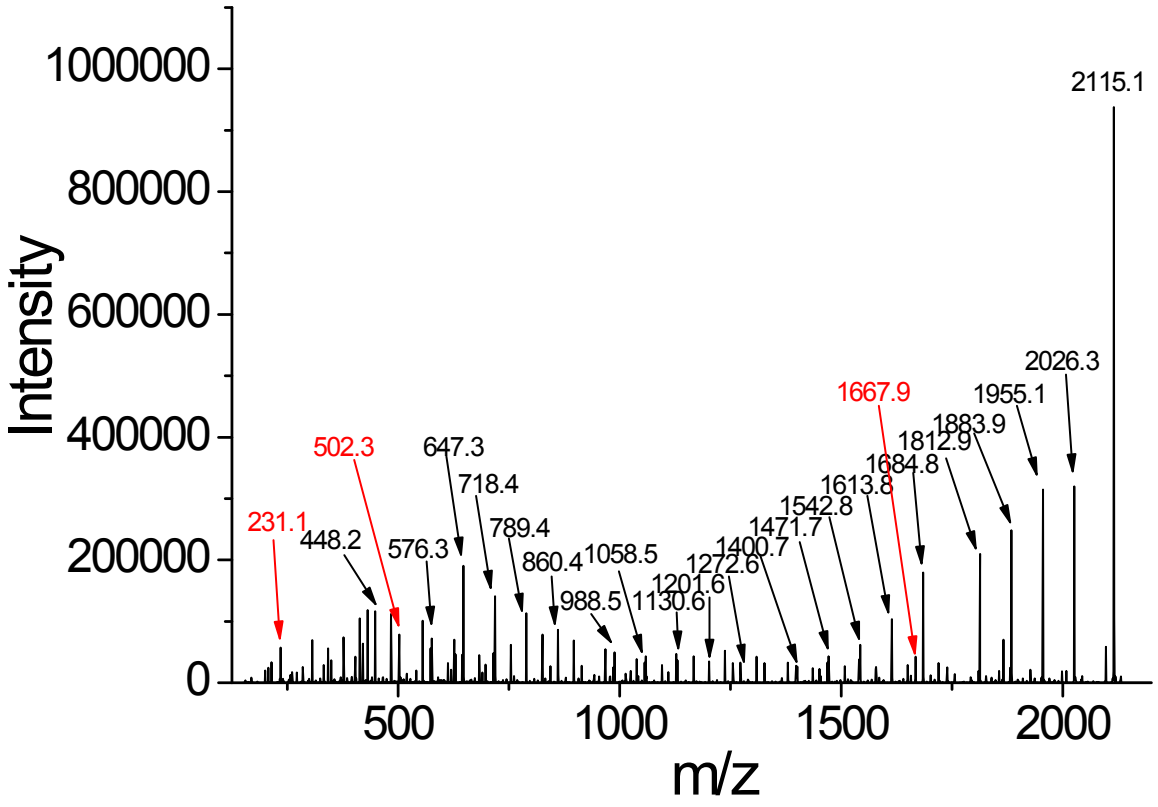

Figure S2. Fragment MS/MS spectrum of unlabeled Pep25 with single proton. The fragment peaks of N-terminal sequences are labeled in black and the peaks of C-terminal sequences are labeled in red. Because of the limitation of space, only three N-terminal sequences are labeled.

Table S1. Fragments of Pep25 with the theoretical formula weight and the detected formula weight in Figure S2.

Fragment of N-terminal sequence	Theoretical m/z	Detected m/z	Complementary C-terminal sequence	Theoretical m/z	Detected m/z
AAAKAAAAKAAAAKAAAAKAAAAY	2026.4	2026.3	A	89.1	N/A
AAKAAAAKAAAAKAAAAKAAAAY	1955.3	1955.1	AA	160.2	N/A
AKAAAAKAAAAKAAAAKAAAAY	1884.2	1883.9	AAA	231.3	231.1
KAAAAKAAAAKAAAAKAAAAY	1813.1	1812.9	AAAA	302.4	303.1
AAAAKAAAAKAAAAKAAAAY	1685.0	1684.8	AAAAK	430.5	431.2
AAAKAAAAKAAAAKAAAAY	1613.9	1613.8	AAAAKA	501.6	502.3
AAKAAAAKAAAAKAAAAY	1542.8	1542.8	AAAAKAA	572.7	573.3

AKAAAAKAAAAKAAAAY	1471.7	1471.7	AAAAKAAA	643.8	644.3
KAAAAKAAAAKAAAAY	1400.7	1400.7	AAAAKAAAA	714.9	715.4
AAAAKAAAAKAAAAY	1272.5	1272.6	AAAAKAAAAK	843.0	843.5
AAAKAAAAKAAAAY	1201.4	1201.6	AAAAKAAAAKA	914.1	914.5
AAKAAAAKAAAAY	1130.3	1130.6	AAAAKAAAAKAA	985.2	985.6
AKAAAAKAAAAY	1059.2	1058.5	AAAAKAAAAKAAA	1056.3	1056.6
KAAAAKAAAAY	988.2	988.5	AAAAKAAAAKAAAA	1127.3	1127.6
AAAKAAAAY	860.0	860.4	AAAAKAAAAKAAAAKA	1255.5	1255.7
AAKAAAAY	788.9	789.4	AAAAKAAAAKAAAAKAA	1326.6	1326.8
AKAAAAY	717.8	718.4	AAAAKAAAAKAAAAKAAA	1397.7	1397.8
KAAAAY	646.8	647.3	AAAAKAAAAKAAAAKAAAA	1468.8	1468.8
AAAAY	575.7	576.3	AAAAKAAAAKAAAAKAAAAK	1539.8	1539.8
AAAY	447.6	448.2	AAAAKAAAAKAAAAKAAAAKA	1668.0	1667.9

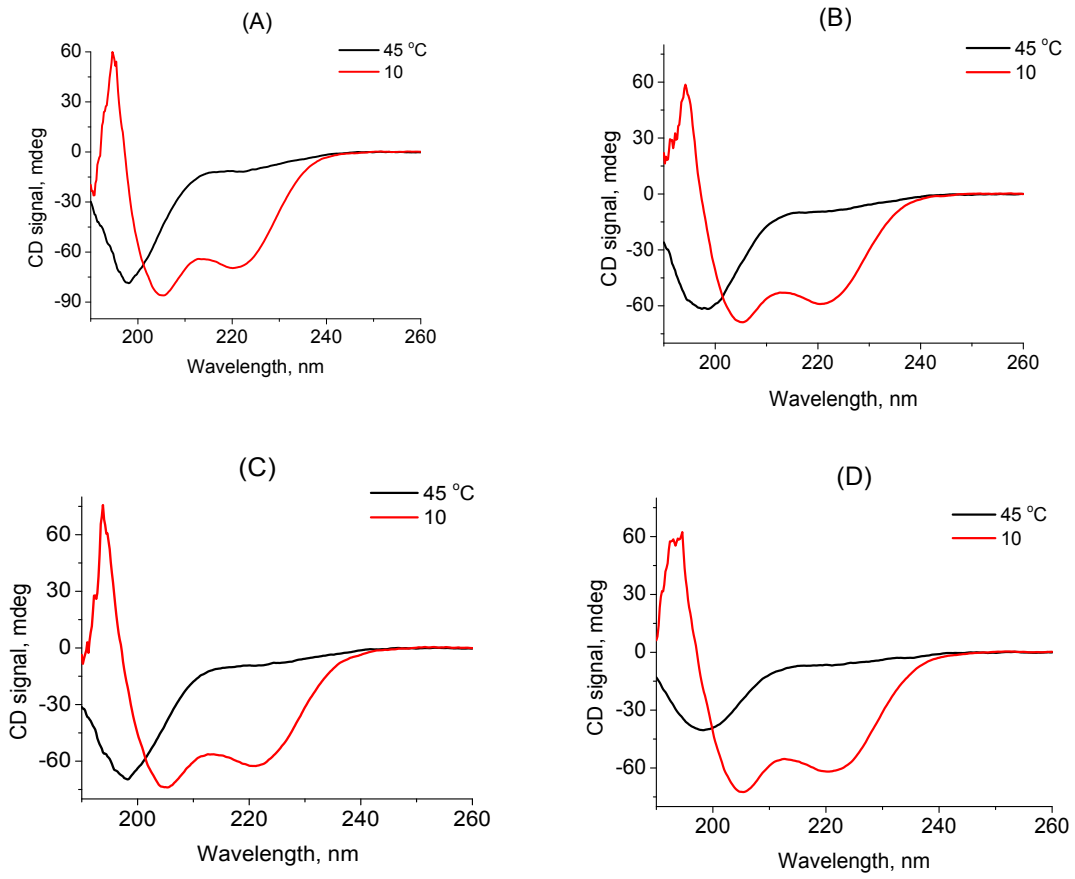

Figure S3. CD spectra of double ^{13}C labeled Pep25 at 10 and 45 ^{o}C: (A) L1, (B) L2, (C) L3, and (D) L4.

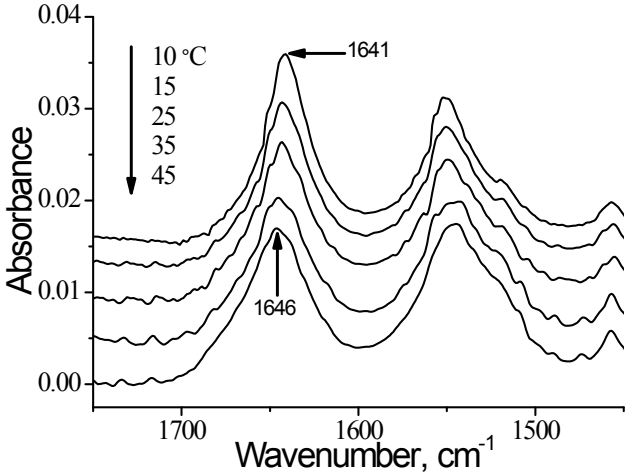

Figure S4. ATR-FTIR spectra of unlabeled Pep25 at various temperatures.

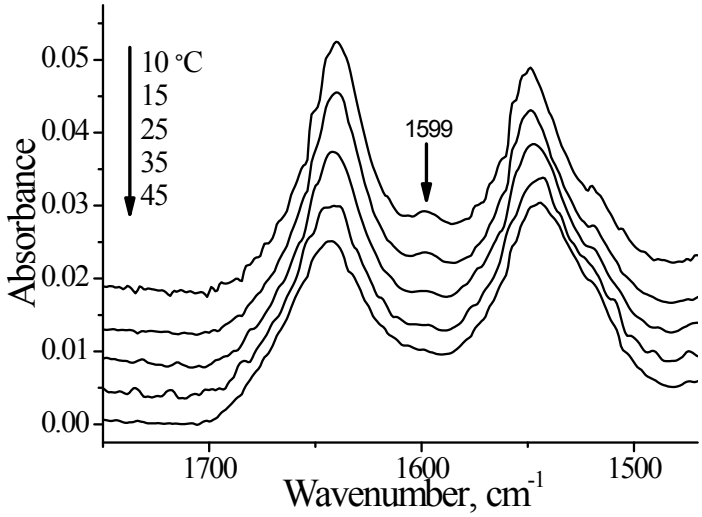

Figure S5. ATR-FTIR spectra of L3 at various temperatures.

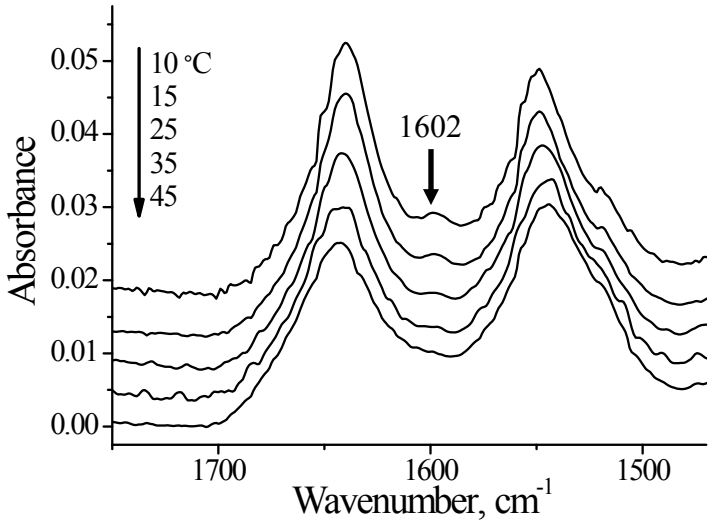

Figure S6. ATR-FTIR spectra of L4 at various temperatures.