Conformational Dynamics and Stability of HP35 Studied with 2D IR Vibrational Echoes

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Supporting Information

NMR Analysis of HP35-P_2

The nature of the HP35-P_2 was explored by NMR spectroscopy, which provides strong evidence that it maintains a folded, native-like structure similar to wild type HP35, and that the two CN's share essentially the same environment within the hydrophobic core of the folded peptide.

NMR experiments were carried out at 25 ºC with 15 mM peptide in 50 mM sodium acetate buffer (D_2O, pD 5.0). 1D ^1H, 2D correlation (COSY), $^1H/^{13}C$ heteronuclear single-quantum correlation (HSQC), and $^1H/^{13}C$ heteronuclear multiple-bond correlation (HMBC) spectra were acquired on a 600 MHz Varian Inova NMR spectrometer with a Varian triple resonance {H,C,N} z-gradient probe. The ^1H spectrum was acquired with 4 scans, an acquisition time of 4 seconds, and a recycle delay of 0.5 seconds. 1D ^{13}C in natural abundance was acquired on a 500 MHz Varian Inova NMR spectrometer with a Varian Switchable {X,H} z-gradient probe. The spectrum was acquired with 11,000 scans with an acquisition time of 2 seconds, a recycle delay of 3 seconds, and a 5 ms (40º) pulse length.

NMR can provide sensitive data about the structure of the peptide and the environment around the CNs. The ^1HNMR of HP35-P_2 is shown in Figure S1 (top panel).

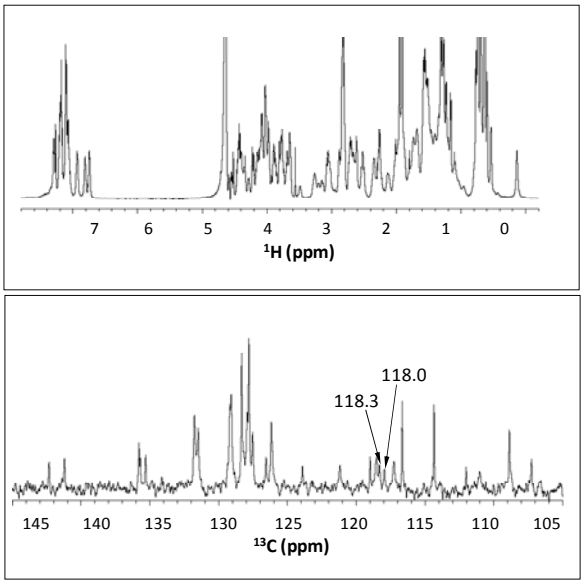

Figure S1. ^1H NMR (top) and the ^{13}C NMR spectra (bottom) of HP35-P_2.

The spectrum shows sharp and well-dispersed peaks, indicating a native-like state rather than unfolded or molten globule structures.[1-3] In addition, the spectrum bears a close resemblance to that of the wild type HP35.[3] Shown also in Figure S1 (bottom panel) is the aromatic region of natural abundance ^{13}CNMR spectrum. The CN carbons were assigned, based on ^{1}H/^{13}C HSQC and ^{1}H/^{13}C HMBC (see below), to the peaks at 118.0 and 118.3 ppm. The NMR relaxation times, T_2^*, are 35 ms and 24 ms, respectively, which are within the expected range for amino acids not exposed to solvent The similarity of the chemical shifts and relaxation times suggest that the two CNs are in very similar environments.

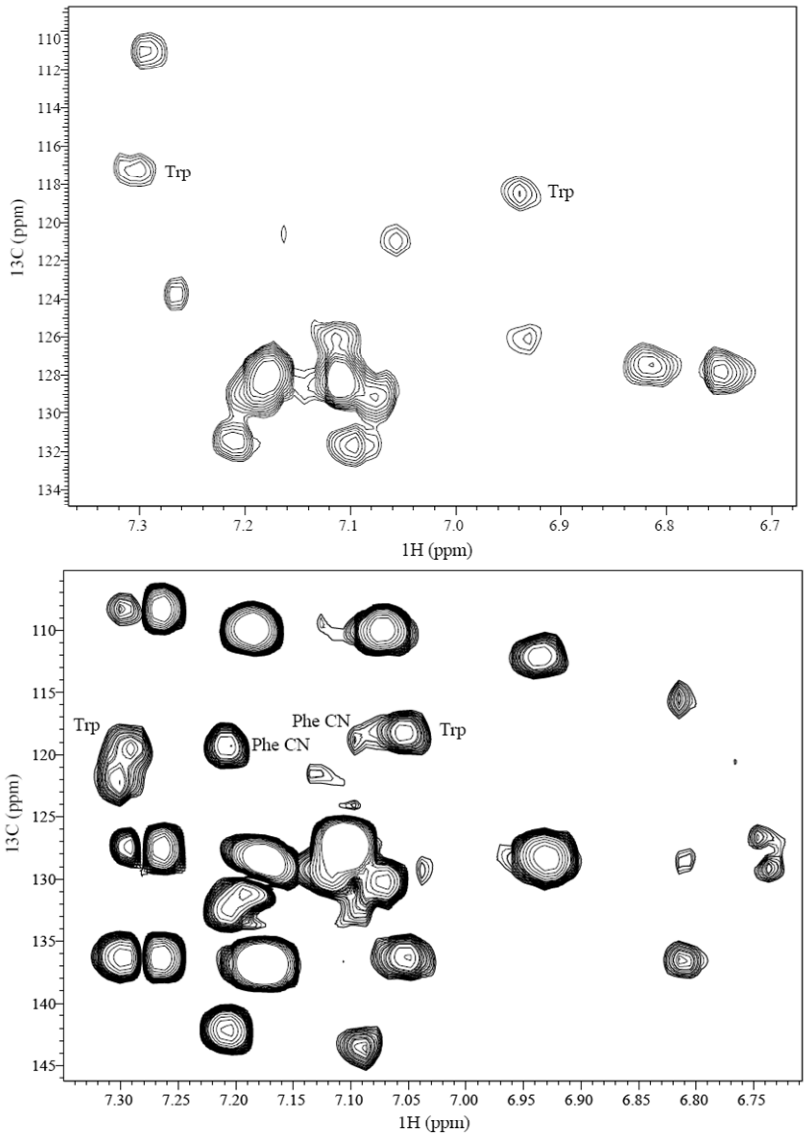

Figure S2 ^{1}H/^{13}C HSQC (top) and HMBC (bottom) NMR spectra of HP35-P_2. The peaks at 118.0 and 118.3 ppm are not apparent in the HSQC, which means that the resonances are not directly attached to ^{1}H. At the same time, they correlate to ^{1}H in the HMBC from Phe and not Trp. These observations indicate that the peaks are those of Phe-attached CN. HSQC spectrum was acquired with 1 scan of 2048 points in t_2 and 100 points in t_1 with an acquisition time of 0.17 s and a recycle delay of 1.5 s. HMBC spectrum was acquired with 32 scans of 2048 points in t_2 and 180 points in t_1 with an acquisition time of 0.17 s and recycle delay of 2 s.

1D ^{13}C (ppm)	HSQC (ppm)	HMBC (ppm)	Assignment	Linewidth (Hz)	T2* (ms)
107.2	-	107.4	W	3.7	86
108.9	-	108.9	F_{CN}	3.5	90
108.9	-	108.9	F_{CN}	3.5	90
111.1	111.3	111.1			
112.0					
114.3		114.6	F (terminal)	0.5	630
116.7				0.4	800
117.3	117.1	117.1	W	20	16
118.0		117.7	CN	9	35
118.3		118.3	CN	13	24
118.6	118.5	118.6	W	23	14

Table S1 Peak assignments from natural abundance 1D ^{13}C, ^{1}H/^{13}C HSQC and HMBC spectra.

References

(1) Baum, J.; Dobson, C. M.; Evans, P. A.; Hanley, C. *Biochemistry* **1989**, *28*, 7-13.
(2) Kiefhaber, T.; Labhardt, A. M.; Baldwin, R. L. *Nature* **1995**, *375*, 513-515.
(3) Gao, J. M.; Kelly, J. W. *Protein Sci.* **2008**, *17*, 1096-1101.

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