

Supplementary material for Dycke et al.

Detailed analysis of phenylalanine signals in NMR spectra

Additional signals of phenyl rings occur with much broader line-widths in both peptides. The corresponding spin-systems are labeled a_{1ii}/a_{2ii} for the truncated peptide in Figure 4A and b_{1ii}/b_{2ii} for the full-length peptide in Figure 4B. The presence of this second set of aromatic rings is independent of the presence of heme (not shown) and indicates that the phenyl rings can adopt two different chemical environments in both peptides. More interestingly, it can be noticed that the relative ratio of the i-type signals versus ii-type is dependent on the peptide length (compare 1D-traces in Figure 4C and 4D). The former is relatively more abundant in the truncated peptide than in the full-length one (Figure 4). Furthermore other spin-systems are only observed with one of the samples: a_{2iii} occurs only in the case of the truncated peptide (Figure 4A), and b_{1iii} in that of the full-length peptide (Figure 4B). Therefore, among the subsets of spin systems observed in each TOCSY spectrum, interaction with heme changes the environment of the two phenylalanines (those labeled with subscripts 1 and 2 in Figure 4) in different ways for both forms of the 73aa-Domain.

Table 1 Identification of TOCSY spin-systems sensitive/insensitive to heme addition on the full-length and truncated 73-D peptide^a

Spin-system	TOCSY correlations disappearing upon heme addition on full-length 73-D		TOCSY correlations common to full-length 73- D and heme-reacted truncated 73-D		Tentative assignment
	$\delta(\text{ppm})^b$	$\delta(\text{ppm})^c$	$\delta(\text{ppm})^d$	$\delta(\text{ppm})^e$	
1	8.31	4.61;2.70;2.55			Asp, Asn
2	8.31	3.96	8.43	3.94	Gly
3	8.25	3.98	8.27	3.94	Gly
4	8.23	2.21	8.25	4.26;2.23	
5	8.20	3.98	8.18	3.97	Gly
6	8.12	2.17			
7	8.10	2.23			
8	8.08	2.75;2.64			Asp, Asn
9	8.07	5.95			
10	7.95	6.09;5.93			
11	7.89	7.08			
12	7.89	4.12;2.26;2.08;1.89			Glu; Gln
13	7.79	7.68			
14	4.47	2.32;2.05;1.97	4.49	3.59;3.45;2.29;2.12;1.88;1.79	Pro
15	4.45	3.69;3.59;1.85	4.23	3.63;3.56	
16	4.21	4.03;3.96			
17	4.20	2.81	4.16	2.59	Asp
18	4.18	3.96			Gly
19	4.17	3.64;2.64			
20	4.17	3.75			
21	4.07	3.66;3.49	4.01	3.47	Ser
22	3.96	2.36;2.04			
23	3.92	3.41;1.44			
24	3.72	2.71;2.65			
25	3.43	2.65			
26	2.96	2.41			
27	2.97	2.27			

^a Some of the spin-systems are not complete due to proton exchange with water or accidental signal overlaps. Spectra were recorded in 50 mM phosphate buffer pH 7 in H₂O:D₂O 90:10 (v:v) at 25°C

^b ¹H chemical shift in the direct dimension of the TOCSY spectrum of the full-length 73-D peptide.

^c ¹H chemical shift in the indirect dimension of the TOCSY spectrum of the full-length 73-D peptide.

^d ¹H chemical shift in the direct dimension of the TOCSY spectrum of the heme-loaded truncated 73-D peptide.

^e ¹H chemical shift in the indirect dimension of the TOCSY spectrum of the heme-loaded truncated 73-D peptide.

Table 2 TOCSY cross peaks present in the heme-reacted truncated 73-D peptide but absent in the full-length 73D peptide with heme^a

Spin-system	δ (ppm) ^b	δ (ppm) ^c	Tentative assignment
1	8.30	4.35;1.65;1.59	Leu, Ile
6	8.24	2.02;1.89	
7	8.25	4.26;2.23	
9	7.95	4.37	
10	7.90	3.71	
11	7.64	6.93	
12	4.16	3.72;3.61;2.77	
13	4.08	3.37;3.28;2.30;1.96	Lys, Pro, (Arg?)
16	4.01	3.47	
18	3.83	1.26	
19	3.52	1.50	
20	3.51	0.85	
22	3.37	2.02	

^a Most of the spin-systems are incomplete due to signal overlaps. Data recorded in 50 mM phosphate buffer pH 7 in H₂O:D₂O 90:10 (v:v) at 25°C

^b ¹H chemical shift in the direct dimension of the TOCSY spectrum of the heme-loaded truncated 73-D peptide.

^c ¹H chemical shift in the indirect dimension of the TOCSY spectrum of the heme-loaded truncated 73-D peptide.

Table 3 New TOCSY cross peaks detected upon heme addition to the full-length 73D peptide^a

Spin-system	δ (ppm) ^b	δ (ppm) ^c	Tentative assignment
1	7.79	7.03	NH ₂ side-chain
2	3.92	2.30;1.64	

^a Most of the spin-systems are incomplete due to signal overlaps or water exchange. Data recorded in 50 mM phosphate buffer pH 7 in H₂O:D₂O 90:10 (v:v) at 25°C

^b ¹H chemical shift in the direct dimension of the TOCSY spectrum of the heme-loaded full-length 73-D peptide.

^c ¹H chemical shift in the indirect dimension of the TOCSY spectrum of the heme-loaded full-length 73-D peptide.

Table 4 Cross peaks present in the TOCSY spectrum of the heme-containing full-length 73-D peptide that disappear in the TOCSY spectrum of the heme-containing truncated 73-D peptide^a

Spin-system	δ (ppm) ^b	δ (ppm) ^c	Tentative assignment
1	8.41	4.45	
2	8.38	4.44	
3	8.35	4.48	
4	8.23	0.89	Leu, Ile
5	8.19	0.90	Leu, Ile
6	8.04	3.73	
7	8.01	6.07	
8	8.01	2.63	
9	7.97	3.92;3.72	
10	7.97	3.80	
11	7.93	4.28	
12	7.79	7.03	
13	7.68	7.49	
14	7.68	7.23	
15	7.68	7.15	
16	7.58	7.19	
17	7.58	7.11	
18	7.49	7.23	
19	7.49	7.14	
20	7.48	5.75	
21	7.45	7.19	
22	7.45	7.11	
23	4.40	3.13	
24	4.33	3.98	
25	4.19	1.00	
26	4.15	1.81	
27	4.13	1.67	
28	3.90	2.30	
29	3.88	1.98 ; 1.66	
30	3.61	3.10 ; 2.90	

^a All the spin-systems are incomplete due to signal overlaps. Data recorded in 50 mM phosphate buffer pH 7 in H₂O:D₂O 90:10 (v:v) at 25°C

^b ¹H chemical shift in the direct dimension of the TOCSY spectrum of the heme-loaded full-length 73-D peptide.

^c ¹H chemical shift in the indirect dimension of the TOCSY spectrum of the heme-loaded full-length 73-D peptide.