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Complete NMR Spectroscopic Assignment of a Neuronal Transduction Protein

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Summary. PDZ domains are found in a wide array of proteins possessing various biological functions like clustering membrane proteins, organizing signal transduction complexes, and maintaining cell polarity. This report presents a complete chemical shift assignment of the PDZ domain of neuronal nitric oxide synthase. The secondary structure based on C_α and C_β chemical shifts is presented.

Keywords. Heteronuclear NMR; Chemical shift assignment; Protein; Structure.

Introduction

Ion channels and receptors, which are localized on the cell surface, are designed for specific functions. For example, at the neuromuscular junction, the release of acetylcholine receptor from the nerve terminal initiates muscle cell depolarization and contraction. Similarly, in the central nervous system, localization of specific ion channels and other membrane-associated proteins at the pre- and postsynaptic membranes is essential for the proper transfer of electric signals from one neuron to the next.

Recently, a novel modular domain coined PDZ domain has been identified in proteins typically associated with cell junctions, including synapses of the central nervous system. PDZ domains are also found in different domain contexts in a variety of other proteins, including protein tyrosine phosphatase [1], a putative calmodulin-dependent kinase [2], and neuronal nitric oxide synthase (n*NOS*) [3].

An important function of PDZ domains was implicated when the two N-terminal PDZ domains (PDZ-1 and PDZ-2) of PSD-95 and its close relatives were shown to act as specific binding modules for the peptide motif *Thr/Ser-X-Val* found at the C-terminus of shaker-type K^+ channels [4] and of NR2 subunits of N-methyl-*D*-aspartic acid (*NMDA*) receptor ion channels [5]. The interaction between the PDZ domains of PSD-95 and the C-terminus of K^+ channel subunits results in the coclustering of both proteins when they are coexpressed in heterologous cells.

PDZ domains have thus recently emerged as novel modules for specific protein–protein binding, important not only in the clustering of membrane proteins, but also

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Table 1. ^{1}H chemical shifts (ppm) of n*NOS* PDZ (100 m*M*) at 35°C in phosphate buffer; the shifts of the first four residues were not identified

Sequence	Residue	NH	αH	βH	γ_1H	γ_2H	δ_1H	δ_2H	ε_1H	ε_2H	δ NH$_2$
4	*Met*	8.42									
5	*Ile*	8.23	4.16	1.85	1.46, 1.21	0.9	0.85				
6	*Glu*	8.32	4.65	2.12, 1.90	2.35, 2.34						
7	*Pro*		4.5	2.10, 2.35			3.73	3.82			
8	*Asn*	8.81	4.86	3.03							7.37, 6.76
9	*Val*	7.6	5.18	1.88	0.85	0.78					
10	*Ile*	9	4.9	1.64	1.27	0.76	0.65				
11	*Ser*	9.28	5.39	4.45							
12	*Val*	8.98	4.33	1.82	0.88	0.81					
13	*Arg*	8.47	5.39	1.83	1.47		3.01				
14	*Leu*	9.19	4.901	1.47	1.63		0.95	0.93			
15	*Phe*	8.46	4.54	2.61, 2.91			6.13		7.01	7.01	
16	*Lys*	7.76	3.69	1.72							
17	*Arg*	7.4	4.21	1.8, 2	1.77		3.28				
18	*Lys*	9.23	3.93	1.87	1.44	0.89	1.69		2.96		
19	*Val*	8.04	4.1	2.05	0.89						
20	*Gly*	8.6	4.34, 3.56								
21	*Gly*	7.46	4.02, 3.69								
22	*Leu*	11.24	4.22		1.54		0.74	0.76			
23	*Gly*	8.85	4.25, 4								
24	*Phe*	7.99	5.19	3.02, 3.45			6.79		6.81	6.81	
25	*Leu*	8.71	5.39	1.69	1.49	0.98	0.82	0.81			
26	*Val*	9.23	5.49	2.34	0.93						
27	*Lys*	8.87	5.11	1.76	1.4	2.05	1.57		2.83		
28	*Glu*	7.71	4.49	0.58, 1.40	2.05	1.62					
29	*Arg*	8.65	4.53	1.84	1.7	0.98	3.13				
30	*Val*	8.28	4.01	2.18	0.96						
31	*Ser*	7.39	4.52	3.79, 4.12		1.45					
32	*Lys*	8.05	4.087	1.68	1.53	1.94	1.72		2.95		

(*continued*)

Table 1 (*continued*)

Sequence	Residue	NH	αH	βH	γ_1H	γ_2H	δ_1H	δ_2H	ε_1H	ε_2H	δ NH$_2$
33	*Pro*		4.85	2.13, 2.46	1.84	2.02	3.52	3.52			
34	*Pro*		4.91	2.24, 2.47	1.89	0.87	3.65	3.88			
35	*Val*	8.3	4.82	2.2	0.87	0.8					
36	*Ile*	8.67	4.98	1.34	1.47, 1	0.5	0.83				
37	*Ile*	8.78	4.21	1.81	1.39, 0.34		−0.12				
38	*Ser*	9.57	4.46	3.76, 3.46							
39	*Asp*	7.63	4.78	2.57, 2.43							
40	*Leu*	9.01	4.74	1.85, 1.4	1.64	0.87	0.62	0.83			
41	*lle*	7.57	4.07	1.6	1.05, 1.34	1.62	0.75				
42	*Arg*	9.06	4.21	1.82	1.67		3.28				
43	*Gly*	9.78	4.21, 3.76								
44	*Gly*	7.9	4.26, 3.93								
45	*Ala*	9.38	4.08	1.67							
46	*Ala*	8.18	4.05	1.52		2.28					
47	*Glu*	10.13	3.89	2.13, 2.13	2.28	2.39					
48	*Gln*	8.42	4.04	2.12, 1.94	2.59					6.76, 7.64	
49	*Ser*	7.84	4.26	4.02, 4.12							
50	*Gly*	7.29	4.21, 4								
51	*Leu*	7.95	4.31	1.46, 1.71	1.72	0.54	0.97	0.67			
52	*Ile*	7.44	3.96	1.23	1.44, 0.93	2.1	0.69				
53	*Gln*	8.9	4.5	1.67, 1.85	1.92						6.32, 7.14
54	*Ala*	8.46	3.64	1.19							
55	*Gly*	9.16	4.4, 3.37								
56	*Asp*	7.59	4.72	2.1, 2.46		0.81					
57	*Ile*	8.72	4.52	1.8	1.22, 1	0.77	0.85				
58	*Ile*	7.16	4.17	1.62	1.47, 1		0.75				
59	*Leu*	9.3	4.37	1.51, 1.42	1.64		0.75	0.86			
60	*Ala*	7.98	4.92	1.18		0.81					
61	*Val*	8.13	4.6	1.78	0.81						
62	*Asn*	9.9	4.3	3.05							7.09, 7.8

(*continued*)

Table 1 (*continued*)

Sequence	Residue	NH	αH	βH	γ_1H	γ_2H	δ_1H	δ_2H	ε_1H	ε_2H	δ NH_2
63	*Asp*	8.88	4.35	2.77, 2.97		1.62					
64	*Arg*	8.46	4.88	1.84	1.7	2.02	3.13				
65	*Pro*		4.75	2.10, 2.45	1.89		3.9	4.14			
66	*Leu*	8.75	4.6	1.46, 1.71	1.57	1.04	0.76	0.77			
67	*Val*	7.07	3.77	2.28	1.08						
68	*Asp*	8.56	4.94	2.83, 2.45							
69	*Leu*	7.18	4.56	1.67, 1.64	1.7		0.81	0.82			
70	*Ser*	8.25	4.58	4.4, 4.08							
71	*Tyr*	9.31	3.83	2.93, 3.14			6.98		6.52	6.52	
72	*Asp*	8.61	4.12	2.64, 2.52							
73	*Ser*	7.9	4.26	3.93, 4.12							
74	*Ala*	8.71	3.87	1.34							
75	*Leu*	8.57	3.72	1.43, 1.23	1.23	2.29	0.53	0.53			
76	*Glu*	7.42	3.93	2.13, 2.07	2.37						
77	*Val*	7.52	3.57	2.26	0.95						
78	*Leu*	7.8	3.81	1.96, 1.64	1.24		0.84	0.84			
79	*Arg*	8.79	4.06	1.8	1.64		3.09		6.93		
80	*Gly*	7.67	3.97, 3.68								
81	*Ile*	6.68	3.74	1.36	1.69, 1.29		0.9				
82	*Ala*	8.67	4.12	1.36							
83	*Ser*	8.42	3.7	3.5, 4.12							
84	*Glu*	8.38	3.18	2.19, 2.09	2.42						
85	*Thr*	6.52	4.52	4.06							
86	*His*	8.48	5.28	2.85, 3							6.92
87	*Val*	9.48	4.75	1.95	0.89						
88	*Val*	8.28	4.78	1.99	0.77						
89	*Leu*	9.37	5.03	1.28, 1.28	1.69		0.91	0.92			
90	*Ile*	8.14	4.83	1.75	1.47, 1.18		0.71				
91	*Leu*	9.24	5.36	1.18, 1.64	1.47		0.68		0.73		
92	*Arg*	9.14	5.12	2.03	1.73		3.22		7.28		
93	*Gly*	8.57	4.58, 3.57								
94	*Pro*		4.48	1.91, 2.32	2.01		3.58	3.87			

(*continued*)

Table 1 (*continued*)

Sequence	Residue	NH	αH	βH	γ_1H	γ_2H	δ_1H	δ_2H	ε_1H	ε_2H	δ NH$_2$
95	*Glu*	8.47	4.26	1.99, 2.07	2.37						
96	*Gly*	8.94	4.21, 3.56								
97	*Phe*	7.67	5.05	2.91, 2.73			6.85		7.3	7.3	
98	*Thr*	9.47	5.06	4.17							
99	*Thr*	8.59	5.61	3.86							
100	*His*	8.65	4.86	3.32, 3.40							
101	*Leu*	8.87	5.41	1.85, 1.64	1.64		0.91	0.95			
102	*Glu*	8.78	4.04	1.99, 2.07	2.42						
103	*Thr*	9.11	5.14	3.83	0.85						
104	*Thr*	8.77	4.67	4.15							
105	*Phe*	8.42	5.27	2.87			7.15		7.29	7.29	
106	*Thr*	8.61	4.58	3.77							
107	*Gly*	8.82	3.92, 4.05								
108	*Asp*	7.9	4.72	2.63, 2.91							
109	*Gly*	8.19	3.64, 4.26								
110	*Thr*	7.98	4.59	4.3							
111	*Pro*		4.6	1.72, 2.14	1.85		3.85	4.11			
112	*Lys*	8.79	4.52	1.58	1.20, 1.31		1.58		2.87		
113	*Thr*	8.67	5.1	3.9	1.22						
114	*Ile*	9.1	4.58	1.83	1.02, 1.26		0.75				
115	*Arg*	8.63	4.77	1.79	1.19		3.13				
116	*Val*	8.73	4.16	1.01	0.64						
117	*Thr*	8.71	5.36	3.81							
118	*Gln*	9.01	5.21	1.96, 2.14	2.35					6.87, 7.43	
119	*Pro*		4.4	1.87, 2.26	1.96		3.81	4			
120	*Leu*	7.78	4.22	1.31, 1.42	1.29		0.82	0.78			
121	*Gly*	8.14	3.95, 4.14								
122	*Pro*		4.7	1.93, 2.33	2.02		3.58	3.58			
123	*Pro*		4.5	2.29, 1.94	2.02		3.65	3.8			
124	*Thr*	8.1	4.32	4.17							
125	*Lys*	8.24	4.36	1.74, 1.82	1.4		1.57		2.99		
126	*Ala*	8.35	4.39	1.36							
127	*Val*	7.69	4.04	2.06	0.87	0.89					

in linking signalling molecules in a multiprotein complex at specialized membrane sites. Understanding the function and specificity of these protein modules requires detailed knowledge of their structure. Here, the secondary structure of a PDZ domain from neuronal nitric oxide synthase (n*NOS*) is reported.

Results and Discussion

Although PDZ has a molecular mass of 14 kDa, the homonuclear 2D spectra would have been sufficient to identify the backbone as well as the side chain resonances. However, due to presence of many hydrophobic residues (amino acid sequence shown below), PDZ has poor solubility in aqueous media.

GSHMIEPNVI SVRLFKRKVG GLGFLVKERV SKPPVIISDL
IRGGAAEQSG LIQAGDIILA VNDRPLVDLS YDSALEVLRG
IASETHVVLI LRGPEGFTTH LETTFTGDGT PKTIRVTQPL
GPPTKAV

So, it was necessary to use single and/or double labeled samples for heteronuclear 2D and 3D experiments. The ^{1}H-^{15}N-HSQC, ^{1}H-^{15}N-^{1}H HSQC-NOESY, and ^{1}H-^{15}N-^{1}H HSQC-TOCSY experiments were performed with ^{15}N labeled PDZ samples, whereas CT-^{1}H-^{13}C-HSQC, ^{1}H-^{13}C-^{1}H HSQC-NOESY, and HCCH-TOCSY experiments were executed on ^{13}C labeled PDZ samples. CBCANH and CBCACONH experiments were conducted on double labeled PDZ samples to establish the sequential assignment. CT-^{1}H-^{13}C HSQC and HCCH-TOCSY data were used to determine the chemical shifts and to identify or confirm the chemical shifts of side chain protons attached to carbons. The chemical shifts of all amino acid residues are presented in Tables 1–3. ^{3}J-HNHα was measured by a HNCA-J experiment. An analysis of ^{13}C chemical shifts (Table 2) based on the chemical shift index (CSI) method [18] and ^{3}J-HNHα coupling constants (Fig. 1) indicated

Table 2. ^{13}C chemical shifts of n*NOS* PDZ (100 m*M*) at 35°C in phosphate buffer; the shifts of the first four residues were not identified

Sequence	Residue	Cα	Cβ	Cγ_1	Cγ_2	Cδ_1	Cδ_2	Cε
4	*Met*							
5	*Ile*	61.51	38.75	27.77	18.03	13.04		
6	*Glu*	54.04	29.76	34.18				
7	*Pro*	64.33	33					
8	*Asn*	54.3	38.83					
9	*Val*	61.55	34.3	22.62	22.48			
10	*Ile*	62.2	42.88	26.39	19	13.67		
11	*Ser*	56.01	64.76					
12	*Val*	61.05	36.23	22.1	22.1			
13	*Arg*	55.01	32.27	28.54		43.99		
14	*Leu*	53.71	47.49	27.39		25.08	26.49	
15	*Phe*	57.06	41.07					
16	*Lys*	56.81	32.89					
17	*Arg*	56.81	32.13	27.22		43.53		

(*continued*)

Table 2 (*continued*)

Sequence	Residue	Cα	Cβ	$C\gamma_1$	$C\gamma_2$	$C\delta_1$	$C\delta_2$	Cε
18	*Lys*	60.15	33.01	25.52		29.88		42.57
19	*Val*	62.52	32.03	21.23	22.17			
20	*Gly*	45.05						
21	*Gly*	47.41						
22	*Leu*	56.85	42.11			26.34	23.33	
23	*Gly*	47.02						
24	*Phe*	56.52	41.39					
25	*Leu*	53.87	43.59	28.32		25.71	24.71	
26	*Val*	58.83	35.81	24.15	19.14			
27	*Lys*	54.82	37.73	25.29		30.43		42.81
28	*Glu*	56.33	30.73	36.66				
29	*Arg*	55.41	33.08	27.78				
30	*Val*	64.05	31.74	21.55	20.99			
31	*Ser*	57.07	64.97					
32	*Lys*	55.26	33.64	25.18		29.92		42.95
33	*Pro*	61.7	33.7	25.2		50.6		
34	*Pro*	66.37	33.98	28.45		26.96		
35	*Val*	62.85	33.88	23.36				
36	*Ile*	58.53	43.5	29.95	16.77	15.24		
37	*Ile*	63.3	37.78	28.44	17.34	13.5		
38	*Ser*	59.59	65.32					
39	*Asp*	54.25	45.57					
40	*Leu*	54.27	43.76	28.75		24.58	25.83	
41	*lle*	60.46	38.11	28.02	17.28	11.95		
42	*Arg*	58.28	29.59	27.3		43.53		
43	*Gly*	45.77						
44	*Gly*	45.42						
45	*Ala*	55.93	18.6					
46	*Ala*	55.94	20.02					
47	*Glu*	60.68	30.36	30.49				
48	*Gln*	59.16	28.6	35.12				
49	*Ser*	61.49	64.71					
50	*Gly*	46.97						
51	*Leu*	55.24	44.6	26.69		26.47	23.44	
52	*Ile*	60.25	40.72	27.84	18.39	14.43		
53	*Gln*	54.02	32.77	32.41				
54	*Ala*	54	18.14					
55	*Gly*	44.7						
56	*Asp*	55.79	42.33					
57	*Ile*	60.62	41.5	26.9	18.49	14.34		
58	*Ile*	62.53	38.2		19.74	14.87		
59	*Leu*	56.15	42.9	27.2		23.3	26.75	
60	*Ala*	52.83	22.68					
61	*Val*	60.39	34.51	21.46	21.46			
62	*Asn*	55.3	37.24					
63	*Asp*	55.47	40.52					
64	*Arg*	53.01	31.18	27.78		44.02		

(*continued*)

Table 2 (*continued*)

Sequence	Residue	Cα	Cβ	$C\gamma_1$	$C\gamma_2$	$C\delta_1$	$C\delta_2$	Cε
65	*Pro*	62.84	33.15	28.45		51.8		
66	*Leu*	54.32	43.2	28.57		25.86	23.66	
67	*Val*	66.22	32.64	22.26	21.1			
68	*Asp*	54.26	41.94					
69	*Leu*	54.44	44.41	27.64		26.47	23.11	
70	*Ser*	57.62	65.31					
71	*Tyr*	63.71	39.21					
72	*Asp*	58.05	40.78					
73	*Ser*	62.07	63.46					
74	*Ala*	55.5	19.08					
75	*Leu*	57.73	41.15	27.36		26.54	24.5	
76	*Glu*	59.81	29.64	36.62				
77	*Val*	66.53	31.5	22.58	21.83			
78	*Leu*	58.42	43.15			26.43	26.43	
79	*Arg*	59.23	31	29.5		44		
80	*Gly*	45.84						
81	*Ile*	61.23	38.58	27.63	18.01	14.12		
82	*Ala*	53.34	19.15					
83	*Ser*	60.17	64.22					
84	*Glu*	57.72	26.62	36.66				
85	*Thr*	60.27	73.01		22.11			
86	*His*	57.92	31.96					
87	*Val*	59.78	35.39	21.69	23.06			
88	*Val*	61.87	33.34	21.85	21.6			
89	*Leu*	53.61	44.7	27.63		25.67	27.33	
90	*Ile*	60.54	37.13	27.22	18.56	11.89		
91	*Leu*	53.19	45.58	27.22		26.77	27.01	
92	*Arg*	54.59	34.11		28.7	44.84		
93	*Gly*	44.92						
94	*Pro*	63.1	32.58	27.55		50.08		
95	*Glu*	57.67	30.47	36.83				
96	*Gly*	45.37						
97	*Phe*	56.85	43.37					
98	*Thr*	59.94	70.78	21.34				
99	*Thr*	61.47	72.28		20.42			
100	*His*	55.78	31.96					
101	*Leu*	57.82	42.61			26.54	25.58	
102	*Glu*	60.55	29.56	36.66				
103	*Thr*	61.9	70.9	22.52				
104	*Thr*	59.9	71.37		21.22			
105	*Phe*	57.38	42.45					
106	*Thr*	61.56	70.94		21.75			
107	*Gly*	47.39						
108	*Asp*	53.55	41.04					
109	*Gly*	45.89						
110	*Thr*	61.53	69.63		22.22			
111	*Pro*	62.84	32.75			51.6		

(*continued*)

Table 2 (*continued*)

Sequence	Residue	Cα	Cβ	$C\gamma_1$	$C\gamma_2$	$C\delta_1$	$C\delta_2$	Cε
112	*Lys*	55.79	35.48	25.06		29.66		42.78
113	*Thr*	62.89	70.22		23.9			
114	*Ile*	59.48	42.45	26.83	18.47	14.18		
115	*Arg*	55.16	32.97	27.78				
116	*Val*	62.09	33.93	20.52	21.45			
117	*Thr*	62.35	71.79		23.03			
118	*Gln*	53.13	32.22	34.04				
119	*Pro*	63.45	32.38	28.45		51.3		
120	*Leu*	55.71	43.07	27.68		24.11	25.51	
121	*Gly*	44.8						
122	*Pro*	61.49	31.5	27.7		50.1		
123	*Pro*	63.49	32.37	27.92		50.87		
124	*Thr*	62.06	70.47		22.31			
125	*Lys*	56.35	33.95	25.29		29.63		42.81
126	*Ala*	52.96	19.7					
127	*Val*	63.86	33.94	20.62	22.17			

Table 3. ^{15}N chemical shifts of n*NOS* PDZ (100 m*M*) at 35°C in phosphate buffer; the shifts of the first four residues were not identified

Sequence	Residue	N	δN	εN	ηN
4	*Met*	121.76			
5	*Ile*	123.04			
6	*Glu*	125.12			
7	*Pro*				
8	*Asn*	116.83	113.3		
9	*Val*	119.2			
10	*Ile*	121.48			
11	*Ser*	123.11			
12	*Val*	123.38			
13	*Arg*	127.94			
14	*Leu*	126.77			
15	*Phe*	124.86			
16	*Lys*	125.85			
17	*Arg*	128.21			
18	*Lys*	124.05			
19	*Val*	117.62			
20	*Gly*	114.93			
21	*Gly*	109.47			
22	*Leu*	131.73			
23	*Gly*	105.97			
24	*Phe*	117.8			
25	*Leu*	119.59			
26	*Val*	115.68			
27	*Lys*	117.58			

(*continued*)

Table 3 (*continued*)

Sequence	Residue	N	δN	εN	ηN
28	*Glu*	122.04			
29	*Arg*	126.61			
30	*Val*	118.67			
31	*Ser*	114.96			
32	*Lys*	117.86			
33	*Pro*				
34	*Pro*				
35	*Val*	118.46			
36	*Ile*	126.68			
37	*Ile*	125.39			
38	*Ser*	126.83			
39	*Asp*	116.56			
40	*Leu*	120.71			
41	*Ile*	121.87			
42	*Arg*	133.01			
43	*Gly*	116.82			
44	*Gly*	106.87			
45	*Ala*	121.84			
46	*Ala*	120.14			
47	*Glu*	125.61			
48	*Gln*	117.71		113.3	
49	*Ser*	113.26			
50	*Gly*	108.75			
51	*Leu*	118.8			
52	*Ile*	118.22			
53	*Gln*	126.87		110.1	
54	*Ala*	123.39			
55	*Gly*	113.58			
56	*Asp*	122.04			
57	*Ile*	121.23			
58	*Ile*	123.63			
59	*Leu*	126.07			
60	*Ala*	120.4			
61	*Val*	118.56			
62	*Asn*	128.17	113.3		
63	*Asp*	116.14			
64	*Arg*	123.36			
65	*Pro*				
66	*Leu*	121.75			
67	*Val*	113.51			
68	*Asp*	117.8			
69	*Leu*	120.76			
70	*Ser*	114.39			
71	*Tyr*	122.87			
72	*Asp*	116.33			
73	*Ser*	116.82			
74	*Ala*	126.57			

(*continued*)

Table 3 (*continued*)

Sequence	Residue	N	δN	εN	ηN
75	*Leu*	119.72			
76	*Glu*	118.48			
77	*Val*	120.76			
78	*Leu*	118.84			
79	*Arg*	117.79			85.01
80	*Gly*	105.77			
81	*Ile*	120.84			
82	*Ala*	133.28			
83	*Ser*	116.76			
84	*Glu*	116.53			
85	*Thr*	106.46			
86	*His*	118.38			
87	*Val*	123.45			
88	*Val*	125.6			
89	*Leu*	129.41			
90	*Ile*	122.69			
91	*Leu*	127.92			
92	*Arg*	121.82			85.01
93	*Gly*	111.9			
94	*Pro*				
95	*Glu*	121.53			
96	*Gly*	110.47			
97	*Phe*	118.34			
98	*Thr*	115.58			
99	*Thr*	122.33			
100	*His*	120.86			
101	*Leu*	119.83			
102	*Glu*	120.51			
103	*Thr*	121.7			
104	*Thr*	120.85			
105	*Phe*	119.24			
106	*Thr*	113.72			
107	*Gly*	108.95			
108	*Asp*	118.02			
109	*Gly*	108.3			
110	*Thr*	119.09			
111	*Pro*				
112	*Lys*	124.02			
113	*Thr*	124			
114	*Ile*	122.43			
115	*Arg*	123.19			
116	*Val*	129.37			
117	*Thr*	126.68			
118	*Gln*	125.77		112.3	
119	*Pro*				
120	*Leu*	123.04			
121	*Gly*	109.86			

(*continued*)

Table 3 (*continued*)

Sequence	Residue	N	δN	εN	ηN
122	*Pro*				
123	*Pro*				
124	*Thr*	114.86			
125	*Lys*	124.05			
126	*Ala*	127.29			
127	*Val*	123.61			

the presence of two α-helical regions (residues 45–49 and 71–80) and seven anti-parallel β-sheets (residues 11–14, 25–30, 35–40, 56–60, 85–92, 97–101, and 116–119).

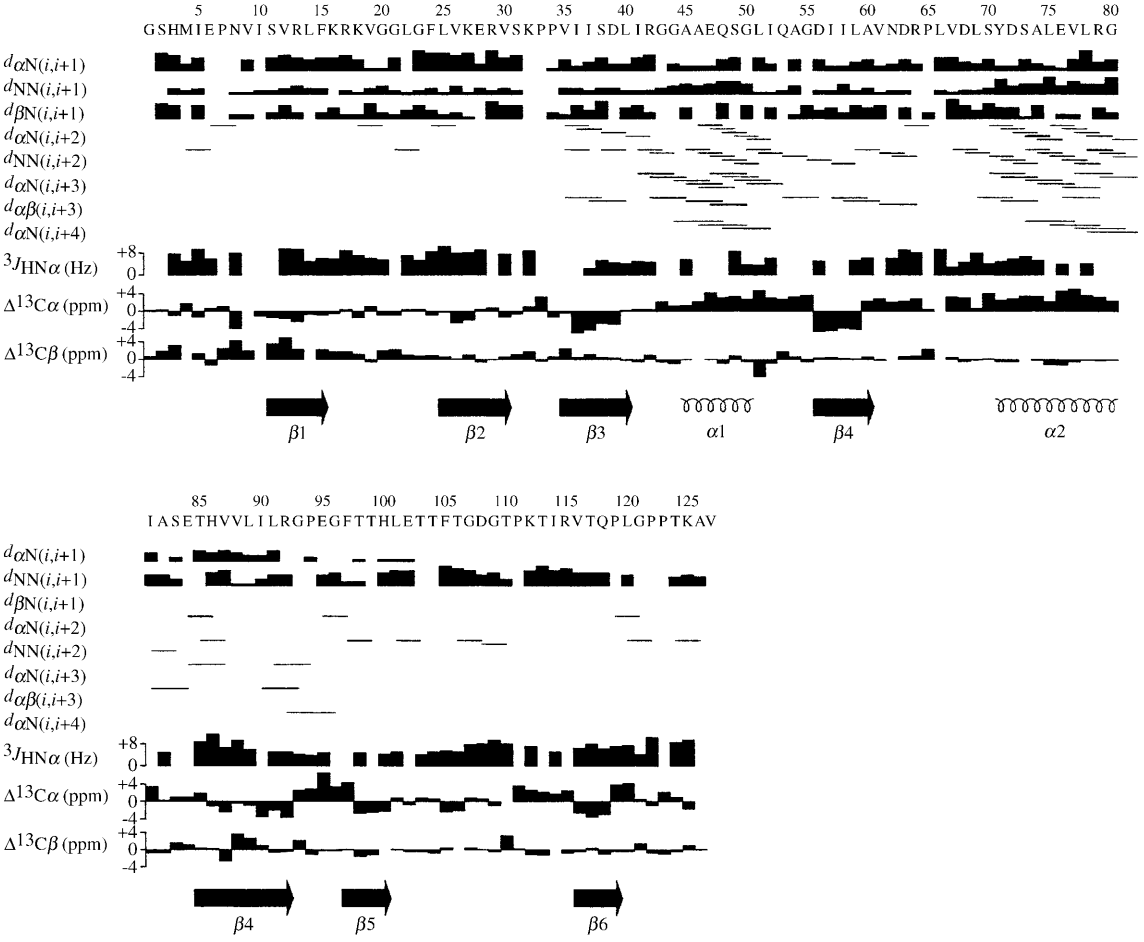

Fig. 1. Chemical shift index plot of the PDZ domain of neuronal nitric oxide synthase; the protein contains two α-helices (coils) and seven β-sheets (arrows); the thickness of the lines corresponds to the relative intensities of NOEs

Experimental

Uniformly ^{15}N and ^{13}C labeled n*NOS* PDZ samples were prepared as mentioned in Refs. [6, 7]. All NMR samples were prepared in a 100 m*M* phosphate buffer ($pH = 6.0$) with D_2O (5% by volume) added for deuterium lock. A trace amount of NaN_3 was added as a preservative against bacterial degradation. The sample concentration was 1.5 m*M*. Dissolved O_2 was removed by slowly passing dry N_2 gas through the sample for 15 minutes, and the tube was sealed. NMR data were recorded at 35°C on a Varian INOVA 500 MHz instrument equipped with *z*-gradients and a triple resonance probe. ^1H and ^{13}C chemical shifts were referenced to *DSS* (2,2-dimethyl-2-silapentane-5-sulfonate, 0 ppm). Indirect referencing was adopted for determining ^{15}N chemical shifts using the frequency ratio $\omega_N/\omega_H = 0.101329118$ [8]. The spectra were processed with nmrPipe [9] and analyzed using the PIPP program [10] on a silicon graphics work station. ^1H, ^{15}N, and ^{13}C backbone and side-chain resonance assignments were obtained using heteronuclear 2D and 3D experiments (^1H-^{15}N-HSQC [11], ^1H-^{13}C-HSQC [11], TOCSY-HSQC [12], NOESY-HSQC [12], CT-HSQC [13], CBCACONH [14], CBCANH [14], HCCH-TOCSY [15–16], and HNCA-J [17]).

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References

[1] Sato T, Irie S, Reed JC (1995) Science **268**: 411
[2] Hata Y, Butz S, Sudhof TC (1996) J Neurosci **15**: 2488
[3] Hall AV, Antoniou H, Wang Y, Cheung AH, Arbus AM, Olson SL, Lu WC, Kau CL, Marsden PA (1994) J Biol Chem **269**: 33082
[4] Kim E, Niethammer M, Rothschild A, Jan YN, Sheng M (1995) Nature **378**: 85
[5] Niethammer M, Kim E, Sheng M (1996) J Neurosci **16**: 2157
[6] Torchi H, Okhi S, Zhang Q, Li M, Zhang M (1998) Nature Structure Biology **5**: 965
[7] Tochio H, Mandal PK, Zhang M (1999) Nature Structure Biology **6**: 417
[8] Wishart DS, Bigag CG, Yao J, Abilgaard F, Dyson HJ, Olefield E, Markley JL, Sykes BD (1995) J Biomolecular NMR **6**: 135
[9] Delaglio F, Grzessiek S, Vuister G, Zhu G, Pfeifer J, Bax A (1995) J Bimolecular NMR **6**: 277
[10] Garrett DS, Powers R, Gronenborn AM, Clore GM (1991) J Mag Reson **95**: 214
[11] Cavanagh J, Rance M (1993) Ann Repor NMR Spectro **27**: 1
[12] Vuister GW, Bax A (1992) J Mag Reson **98**: 428
[13] Kay LE, Ikura M, Tschudin R, Bax A (1990) J Mag Reson **89**: 496
[14] Grzesiek S, Bax A (1992) J Mag Reson **96**: 432
[15] Muhandiram DR, Kay LE (1994) J Mag Reson Ser B **103**: 203
[16] Grzesiek S, Bax A (1992) J Am Chem Soc **114**: 6291
[17] Vuister GW, Bax A (1993) J Am Chem Soc **115**: 7772
[18] Wishart DS, Sykes BD (1994) Methods Enzymol **239**: 363

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