

The complex-formation behaviour of His residues in the fifth Cu²⁺ binding site of human Prion protein: a close look

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SUPPLEMENTARY DATA

Table S1.

Thermodynamic parameters for protonation of PrP₉₁₋₁₁₄, PrP₉₂₋₁₀₀, PrP₁₀₆₋₁₁₃, in aqueous solution. $T = 298$ K, $I = 0.1$ mol dm⁻³ (KCl or KNO₃). Standard deviations are given in parentheses. Charges are omitted for clarity.

Data from F. Berti *et al.*, Chem. Eur. J. 13 (2007) 1991 and M. Remelli *et al.*, Dalton Trans. (2005) 2876.

Species	log β	log K_{step}	$-\Delta H^\circ$ kJ mol ⁻¹	ΔS° J K ⁻¹ mol ⁻¹
PrP ₉₁₋₁₁₄				
HL	11.15	11.15	-	-
H ₂ L	21.46	10.31	-	-
H ₃ L	31.49	10.04	-	-
H ₄ L	40.85	9.35	-	-
H ₅ L	47.39	6.54	-	-
H ₆ L	53.11	5.73	-	-
PrP ₉₂₋₁₀₀				
HL	6.38	6.38	25	38
PrP ₁₀₆₋₁₁₃				
HL	10.52	10.52	81	-69
H ₂ L	20.32	9.80	111	16
H ₃ L	26.48	6.16	135	53

Table S2.

Thermodynamic parameters for Cu^{2+} complex-formation of PrP_{91-114} , PrP_{92-100} , $\text{PrP}_{106-113}$, in aqueous solution. $T = 298 \text{ K}$, $I = 0.1 \text{ mol dm}^{-3}$ (KCl or KNO_3). Standard deviations are given in parentheses. Charges are omitted for clarity.

Data from F. Berti *et al.*, Chem. Eur. J. 13 (2007) 1991 and M. Remelli *et al.*, Dalton Trans. (2005) 2876.

Species	$\log \beta$	$\log K_{\text{step}}$	$-\Delta H^\circ$ kJ mol^{-1}	ΔS° $\text{J K}^{-1} \text{mol}^{-1}$
PrP_{91-114}				
CuH_5L	51.35	5.29	-	-
CuH_4L	46.06	-	-	-
CuH_2L	34.18	6.56	-	-
CuHL	27.62	7.40	-	-
CuL	20.22	8.78	-	-
CuH_{-1}L	11.44	-	-	-
CuH_{-3}L	-8.66	-	-	-
PrP_{92-100}				
CuL	3.85	6.25	20	6
CuH_{-1}L	-2.40	5.82	8	-72
CuH_{-2}L	-8.22	7.55	-39	-27
CuH_{-3}L	-15.77	9.51	-65	-85
CuH_{-4}L	-25.28	-	-88	-187
$\text{PrP}_{106-113}$				
CuH_2L	23.84	6.19	161	-82
CuHL	17.65	5.04	93	27
CuL	12.61	7.71	82	-33
CuH_{-1}L	4.90	9.85	53	-84
CuH_{-2}L	-4.95	10.64	20	-162
CuH_{-3}L	-15.59	11.81	-48	-138
CuH_{-4}L	-27.40	-	-	-

Table S3.

Spectroscopic parameters for Cu²⁺ complex-formation of PrP₉₂₋₁₁₃, PrP₉₂₋₁₀₀, PrP₁₀₆₋₁₁₃ analogues, in aqueous solution. Molar absorptivity is always referred to the total molar concentration of the Cu²⁺ ion. *T* = 298 K, *I* = 0.1 mol dm⁻³ (KCl). Charges are omitted for clarity.

(H96A)PrP ₉₂₋₁₁₃						(H111A)PrP ₉₂₋₁₁₃							
Species (pH)	UV-Vis λ/nm $\epsilon/\text{M}^{-1}\text{cm}^{-1}$		CD λ/nm $\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$		EPR A_{H}/G g_{H}		Species	UV-Vis λ/nm $\epsilon/\text{M}^{-1}\text{cm}^{-1}$		CD λ/nm $\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$		EPR A_{H}/G g_{H}	
CuH ₄ L (pH 5.0)	785	31	292	-0.17	123	2.52	CuH ₄ L (pH 5.5)	795	15	264	0.13	142	2.44
			444	0.11						297	-0.06		
CuH ₂ L (pH 6.5)	617	118	253	5.19	168	2.23	CuH ₃ L (pH 6.0)	780	16	340	0.02	142	2.45
			326	-0.44						526	0.01		
			385	0.14						258	0.62		
			532	0.31						305	-0.08		
CuHL (pH 7.5)	574	133	254	7.05	178	2.22	CuH ₂ L (pH 7.0)	628	55	343	0.13	n.d.	
			317	0.23						514	0.05		
			345	-0.06						626	-0.05		
			390	0.13						271	1.46		
			493	-0.39						322	-0.09		
CuL (pH 9.0)	534	163	644	0.57	192	2.20	CuHL (pH 8.5)	576	97	379	0.11	175	2.22
			257	7.30						489	0.04		
			293	-0.05						616	-0.44		
			318	1.20						276	2.22		
			359	-0.06						335	-0.32		
			404	0.06						391	0.16		
			497	-1.11						612	-0.84		
CuH ₁ L (pH 10.0)	530	175	634	1.10	192	2.20	CuL (pH 9.5)	570	105	275	2.57	204	2.19
			256	7.58						339	-0.28		
			293	-0.11						393	0.16		
			318	1.32						601	-0.95		
			363	-0.06						259	2.59		
			401	0.07						336	-0.18		
CuH ₂ L (pH 10.5)	n.d.	n.d.	635	1.16	n.d.	n.d.	CuH ₁ L (pH 10.0)	566	113	393	0.10	209	2.19
			586	-0.93						586	-0.93		
			257	7.81						253	3.27		
			293	-0.28						316	-0.36		
CuH ₃ L (pH 11.0)	528	175	319	1.32	192	2.20	CuH ₂ L (pH 11.0)	556	121	393	0.05	212	2.19
			399	0.07						581	-0.95		
			498	-1.31						253	3.27		
			637	1.19						316	-0.36		
										393	0.05		
						CuH ₃ L	n.d.		n.d.		n.d.		

(H96His-N^T-Me)PrP₉₂₋₁₁₃						(H111His-N^T-Me)PrP₉₂₋₁₁₃							
pH	UV-Vis		CD		EPR		pH	UV-Vis		CD		EPR	
	λ/nm	$\epsilon/\text{M}^{-1}\text{cm}^{-1}$	λ/nm	$\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$	A_{\parallel}/G	g_{\parallel}		λ/nm	$\epsilon/\text{M}^{-1}\text{cm}^{-1}$	λ/nm	$\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$	A_{\parallel}/G	g_{\parallel}
pH 4.0	803	29	266	0.24	n.d.		pH 4.0	809	29	267	0.18	120	2.42
			292	-0.26						305	-0.10		
pH 5.0	800	33	265	0.28	n.d.		pH 5.0	798	23	264	0.24	120	2.42
			293	-0.20						305	-0.10		
pH 5.5	795	30	255	1.60	n.d.		pH 5.5	768	31	258	1.74	120	2.42
			314	-0.14						317	-0.17		
			425	-0.02						386	0.01		
			527	0.07						523	0.08		
			611	-0.05						610	-0.04		
pH 6.0	632	59	255	4.48	n.d.		pH 6.0	635	51	255	4.74	120	2.42
			317	-0.35						321	-0.31		
			376	0.04						386	0.05		
			435	-0.02						526	0.23		
			525	0.23						613	-0.11		
pH 6.5	619	100	254	6.65	n.d.		pH 6.5	621	101	254	6.23	171	2.22
			316	-0.46						316	-0.34		
			377	0.06						383	0.06		
			434	-0.01						525	0.28		
			524	0.33						606	-0.15		
pH 7.0	611	120	253	6.79	n.d.		pH 7.0	613	115	254	6.43	175	2.22
			313	-0.47						315	-0.29		
			377	0.05						381	0.04		
			433	-0.01						440	-0.02		
			521	0.30						523	0.22		
pH 7.5	602	124	254	6.30	n.d.		pH 7.5	601	121	255	6.11	175	2.22
			310	-0.46						307	-0.23		
			379	0.024						330	-0.09		
			436	-0.02						385	0.02		
			518	0.22						472	-0.06		
pH 8.0	587	130	254	5.90	n.d.		pH 8.0	578	126	255	5.65	175	2.22
			302	-0.47						301	-0.23		
			350	-0.12						327	0.03		
			472	-0.07						353	-0.08		
			517	0.07						478	-0.13		
pH 8.5	562	138	255	5.63	180	2.20	pH 8.5	556	144	256	5.27	179	2.20
			300	-0.40						300	-0.31		
			354	-0.13						325	0.14		
			475	-0.12						357	-0.10		
			574	-0.42						481	-0.21		
pH 9.0	549	147	255	5.43	182	2.20	pH 9.0	547	153	256	5.11	186	2.20
			299	-0.50						298	-0.31		
			327	0.05						325	0.21		
			356	-0.15						358	-0.11		
			475	-0.17						485	-0.25		
pH 10.0	541	170	256	5.23	180	2.18	pH 10.0	539	167	257	5.05	195	2.19
			298	-0.59						297	-0.40		
			326	0.10						324	0.25		
			356	-0.14						361	-0.11		
			481	-0.20						484	-0.26		
pH 11.0	542	173	255	5.38	192	2.20	pH 11.0	536	177	257	5.15	196	2.19
			297	-0.87						297	-0.55		
			330	0.08						326	0.33		
			361	-0.11						365	-0.08		
			480	-0.17						487	-0.31		
			574	-0.63					563	-0.53			
			661	0.31					658	0.33			

(H96His-N^T-Me)PrP₉₂₋₁₀₀						(H111His-N^T-Me)PrP₁₀₆₋₁₁₃									
Species	UV-Vis		CD		EPR		Species	UV-Vis		CD		EPR			
	λ/nm	$\epsilon/\text{M}^{-1}\text{cm}^{-1}$	λ/nm	$\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$	A_{\parallel}/G	g_{\parallel}		λ/nm	$\epsilon/\text{M}^{-1}\text{cm}^{-1}$	λ/nm	$\Delta\epsilon/\text{M}^{-1}\text{cm}^{-1}$	A_{\parallel}/G	g_{\parallel}		
CuH₁L	617	35	259	0.64	119	2.41	CuHL	n.d.	258	2.83	119	2.41			
			306	-0.04					332	-0.34					
			341	0.05					530	0.20					
CuH₂L	600	70	251	3.68	170	2.23	CuL	617	79	249	8.32	170	2.22		
			306	-0.63						337	-0.61				
			346	0.48						388	0.09				
			503	0.40						459	-0.04				
			601	-0.60						536	0.30				
CuH₃L	555	110	247	2.46	180	2.22	CuH₁L	568	100	256	7.47	182	2.21		
			309	-1.48						316	1.15				
			494	0.91						359	-0.11				
			589	-1.46						494	-1.03				
CuH₄L	550	112	246	2.79	196	2.20	CuH₂L	549	108	257	7.65	192	2.20		
			278	0.17						317	1.46				
			310	-1.77						363	-0.10				
			363	-0.04						495	-1.27				
			493	0.80			627	0.94	CuH₃L	543	111	257	7.94	195	2.19
			587	-1.54			319	1.56							
							365	-0.07							
									496	-1.44					
									627	1.03					

Fig. S1

Proton paramagnetic relaxation contributions (R_{1p}) of (H96A)PrP₉₂₋₁₁₃ α protons calculated in presence of 0.1 Cu²⁺ equivalents, T=298 K, pH 6.5 and by using $p_f = 1$ (solid squares) or $p_f = 0.9$ (open circles).

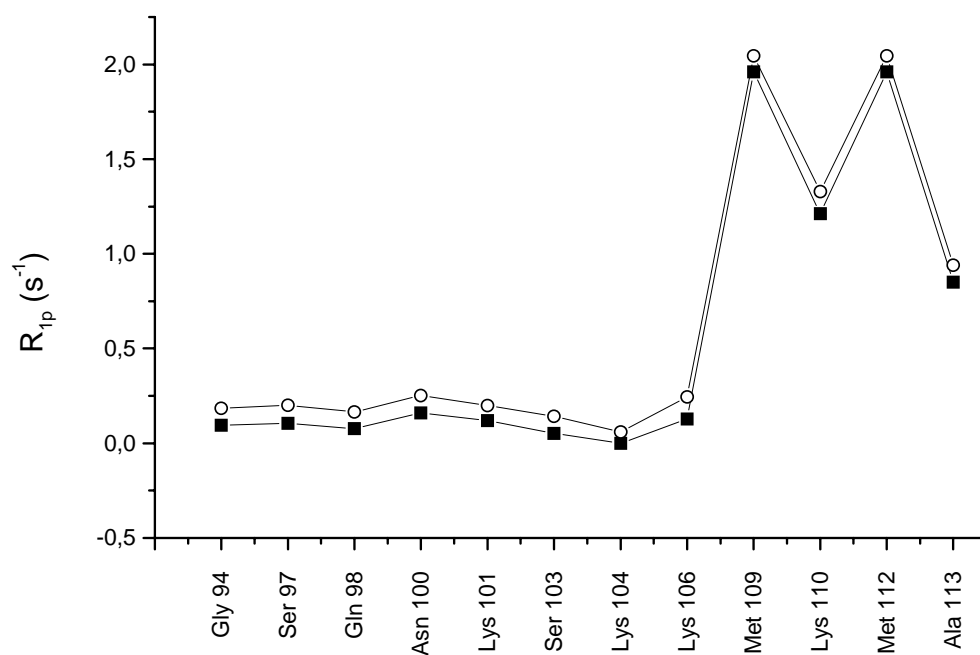


Fig. S2

Comparison between proton paramagnetic relaxation contributions (R_{1p}) of (A) (H96His-N^T-Me)PrP₉₂₋₁₁₃ and (H96His-N^T-Me)PrP₉₂₋₁₀₀; (B) (H111His-N^T-Me)PrP₉₂₋₁₁₃ and (H111His-N^T-Me)PrP₁₀₆₋₁₁₃ calculated in presence of 0.1 Cu²⁺ equivalents, T=298 K, pH 6.5.

