Metal Specificities of *Arabidopsis* Zinc and Copper Transport Proteins Match the Relative, But Not the Absolute, Affinities of their N-Terminal Domains

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Electronic Supplementary Information

Table S1 ESI-MS Data of Isolated Proteins

Protein	Molar Mass (Da)	
	Calculated	**Found**
HMA4n (2-96)	10730	10730
HMA7n (56-127)	8001 [a]	7999 [b]

[a] including extra residues of N-term Met and C-term Trp;

[b] consistent with formation of an intra-molecular disulfide bond.

Table S2. Estimation of K_{ex3} for the competition reaction 3 [a]

3: $[Zn^{II}(Par)_2]$ + Egta $\rightleftharpoons$ Zn^{II}-Egta + 2 Par K_{ex3}

Egta	$[Par]_{total}$ (μM)	$[Zn]_{total}$ (μM)	$[Egta]_{total}$ (μM)	A_{500}	$[Zn(Par)_2]$ (μM)	Θ [b]	K_{ex3} [c] ($x10^{-4}$)
	50.0	10.2	0.00	0.820	10.2		
	49.3	10.1	7.39	0.447	5.58	0.61	4.1
	49.0	10.0	9.80	0.345	4.32	0.58	5.3
	48.8	9.99	12.2	0.269	3.36	0.54	6.3
	48.5	9.95	14.6	0.213	2.66	0.50	7.0
	48.3	9.90	16.9	0.171	2.14	0.46	7.7
	48.1	9.85	19.2	0.139	1.73	0.42	8.4
	47.9	9.80	21.5	0.116	1.46	0.39	8.8
	47.6	9.76	23.8	0.099	1.24	0.36	9.2
	100	10.3	0.00	0.822	10.3		
	98.5	10.1	7.39	0.562	7.02	0.42	7.4
	98.0	10.1	9.80	0.480	5.99	0.42	8.8
	97.6	10.0	12.2	0.415	5.19	0.40	9.6
	97.1	9.98	14.6	0.362	4.52	0.37	10.3
	96.6	9.93	16.9	0.319	3.99	0.35	10.7
	96.2	9.88	19.2	0.284	3.55	0.33	11.0
	95.7	9.84	21.5	0.253	3.16	0.31	11.4
	95.2	9.79	23.8	0.228	2.85	0.29	11.6
	94.8	9.74	26.1	0.207	2.58	0.27	11.8
	94.3	9.70	28.3	0.189	2.36	0.26	11.9
						average	**8.9(2.3)[c]**

[a] The experiments were carried out in Mops buffer (50 mM; pH 7.3; NaCl 100 mM) under anaerobic conditions. The reported values were confirmed with three independent protein samples;

[b] Zn occupancy on Egta;

[c] The observed K_{ex3} for reaction A depends on the Zn occupancy on Egta, suggestive of the possible involvement of tertiary complexes such as Par-Zn-Egta in the reaction. Therefore, the average K_{ex3} must be treated as an approximate indicator of the affinity of Par for Zn^{II} only. However, selection of this data has no influence on the relative difference of protein Zn^{II} K_D obtained via competition with Par given in Table S3 where the observed K_{ex1} for reaction 1 does not depend on the Zn occupancy on the protein and thus is reliable.

Table S3. Estimation of K_{ex1} for competition reaction 1 and of $K_{D(ZnP)}$ from Eq 2 [a]

1: $[Zn^{II}(Par)_2]$ + HMA4n $\rightleftharpoons$ Zn^{II}-HMA4n + 2 Par K_{ex1}

Protein (P)	$[Par]_{total}$ (μM)	$[Zn]_{total}$ (μM)	$[P]_{total}$ (μM)	A_{500}	$[Zn(Par)_2]$ (μM)	Θ [b]	K_{ex1} (x10^{-3})	$K_{D(ZnP)}$ [c] (10^{-10} M)
HMA4n	100	9.33	0	0.747	9.33			
	99.5	9.28	1.84	0.619	7.74	0.84	4.8	
	99.0	9.24	3.66	0.515	6.43	0.77	3.8	
	98.5	9.19	5.47	0.436	5.45	0.68	3.1	
	98.0	9.15	7.25	0.348	4.34	0.66	3.6	
	97.6	9.10	9.02	0.267	3.34	0.64	4.4	
	97.1	9.06	10.8	0.203	2.54	0.60	5.1	
	96.6	9.01	12.5	0.156	1.95	0.56	5.7	
average							4.3(1.2)	**2.1(6)**

[a] The experiments were carried out in Mops buffer (50 mM; pH 7.3; NaCl 100 mM) under anaerobic conditions. The reported values were confirmed with three independent protein samples;

[b] Zn occupancy on Egta;

[c] From Eq 5 where $K_{D(Zn\text{-}Egta)} = 1.0 \times 10^{-9}$ M and $K_{ex3} \sim 8.9 \times 10^{-4}$ (see Table S2) at pH 7.3, NaCl 100 mM.

Table S4. . Estimation of K_{ex1} for competition reaction 1 and of $K_{D(ZnP)}$ from Eq 2 [a]

1: $[Zn^{II}(Par)_2]$ + HMA7n $\rightleftharpoons$ Zn^{II}-HMA7n + 2 Par K_{ex1}

Protein (P)	$[Par]_{total}$ (μM)	$[Zn]_{total}$ (μM)	$[P]_{total}$ (μM)	A_{500}	$[Zn(Par)_2]$ (μM)	Θ [b]	K_{ex1} (x10^{-4})	$K_{D(ZnP)}$ [c] (10^{-9} M)
HMA7n	50.0	9.86	0	0.789	9.86			
	49.8	9.81	3.98	0.627	7.84	0.50	1.5	
	49.5	9.76	7.92	0.527	6.58	0.40	1.3	
	49.3	9.71	11.8	0.455	5.69	0.34	1.3	
	49.0	9.67	15.7	0.391	4.88	0.30	1.4	
	48.8	9.62	19.5	0.344	4.31	0.27	1.4	
	48.5	9.57	23.3	0.297	3.71	0.25	1.5	
	48.3	9.53	27.1	0.269	3.36	0.23	1.5	
	100	9.61	0.00	0.769	9.61			
	100	9.61	3.90	0.718	8.98	0.16	1.5	
	100	9.61	6.50	0.687	8.58	0.16	1.5	
	100	9.61	9.30	0.652	8.15	0.16	1.6	
	100	9.61	13.3	0.615	7.69	0.14	1.6	
	100	9.61	16.6	0.582	7.27	0.14	1.6	
	100	9.61	20.7	0.549	6.86	0.13	1.7	
	100	9.61	25.9	0.521	6.51	0.12	1.6	
	100	9.61	28.8	0.513	6.41	0.11	1.5	
	100	9.61	32.0	0.505	6.31	0.10	1.4	
average							**1.5(1)**	**5.8(4)**

[a] The experiments were carried out in Mops buffer (50 mM; pH 7.3; NaCl 100 mM) under anaerobic conditions. The reported values were confirmed with three independent protein samples;

[b] Zn occupancy on Egta;

[c] From Eq 5 where $K_{D(Zn\text{-}Egta)}$ = 1.0 x 10^{-9} M and K_{ex3} ~ 8.9 x 10^{-4} (see Table S2) at pH 7.3, NaCl 100 mM.

Table S5. Estimation of K_{ex7} for competition reaction 7 and of $K_{D(CuP)}$ from Eq 8 [a]

7: $[Cu^{I}(Bcs)_2]^{3-}$ + *apo*-P $\rightleftharpoons$ Cu^{I}-P + 2 Bcs^{2-} K_{ex7}

Protein (P)	$[Bcs]_{total}$ (μM)	$[Cu^{I}]_{total}$ (μM)	$[P]_{total}$ (μM)	A_{483}	$[Cu(Bcs)_2]^{3-}$ (μM)	$[Cu^{I}P]$ (μM)	Θ [b]	$K_{D(CuP)}$ [c] (M)
HMA4n	200	35.0	3.6	0.4321	33.2	1.8	0.49	3.1×10^{-17}
	200	35.0	7.20	0.413	31.8	3.2	0.45	3.3×10^{-17}
	200	35.0	10.8	0.392	30.2	4.9	0.45	3.0×10^{-17}
	200	35.0	16.1	0.362	27.8	7.2	0.44	2.7×10^{-17}
	200	35.0	20.0	0.342	26.3	8.7	0.43	2.5×10^{-17}
average								**$2.9(3) \times 10^{-17}$**
HMA7n	500	32.2	15.8	0.255	19.6	12.6	0.80	3.7×10^{-19}
	500	32.2	23.7	0.206	15.8	16.4	0.69	5.1×10^{-19}
	500	32.2	31.4	0.174	13.4	18.8	0.60	6.3×10^{-19}
	500	32.2	39.0	0.145	11.2	21.1	0.54	6.6×10^{-19}
	500	32.2	46.6	0.124	9.5	22.7	0.49	6.9×10^{-19}
	500	32.2	54.1	0.118	9.1	23.1	0.43	8.3×10^{-19}
	500	32.2	61.5	0.098	7.5	24.7	0.40	7.6×10^{-19}
	500	32.2	68.9	0.088	6.8	25.4	0.37	7.7×10^{-19}
average								**6.5(1.5) $\times 10^{-19}$**

[a] In Mops buffer (50 mM, pH 7.3; NaCl 100 mM) under anaerobic conditions. The reported are representative values and were confirmed with three independent protein sample preparations.

[b] Occupancy of Cu^{I} on the protein.

[c] From Eq 8 where $\beta_2 = 10^{19.8}$ M^{-2}.

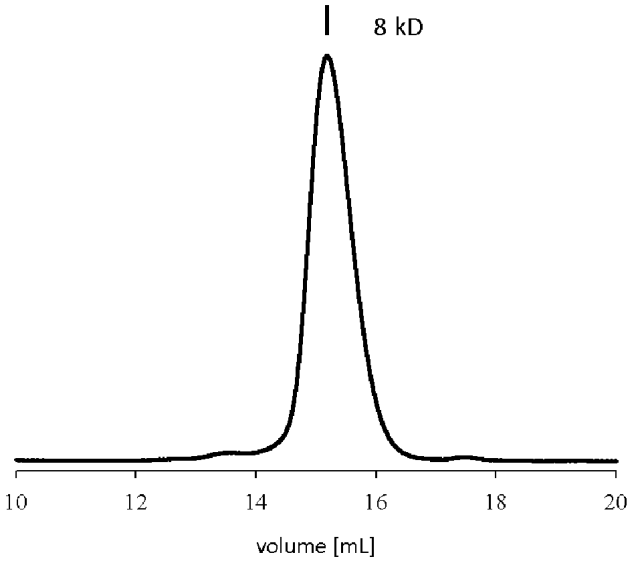

Figure S1. Analytical gel filtration elution profile of Zn-HMA7n on a Superdex-75 gel filtration column (HR10/30; Pharmacia) in Mops buffer (20 mM, pH 7.3, 100 mM NaCl) at a flow rate of 0.7 mL/min.