Characterization of heavy metal binding properties of periplasmic metal uptake protein CLas-ZnuA2

Pranav Kumar,[1] Vikram Dalal,[1] Nidhi Sharma,[1] Sunil Kokane,[2] Dilip Kumar Ghosh,[2] Pravindra Kumar,[1] and Ashwani Kumar Sharma[1]*

[1]Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee-247 667, India.

[2]Plant Virology Laboratory, ICAR-Central Citrus Research Institute, Nagpur-440 010, India.

Supplementary figures and tables

Table S1: Metal coordination distances in Mn-specific available structures of Cluster A-I proteins. The values indicated by * represents the higher than ideal coordination distance.

Protein	Metal ion	Metal coordinating residues						Observed geometry	Ref.
		His39	His106	Glu172		Asp247			
		NE2	NE2	OE1	OE2	OD1	OD2		
CLas-ZnuA2	Cd2+	2.24	2.23	2.23	2.20	2.25	2.23	Octahedral	
CLas-ZnuA2	Mn2+	2.26	2.23	2.31*	2.19	2.22	2.18	Square pyramidal	[1]
	Zn2+	2.08	2.07	2.51*	2.00	2.04	2.03	Square pyramidal	[2]
PsaA	Mn2+	2.11	2.15	2.08	2.39*	2.43*	2.14	Tetrahedral	[3]
	Zn2+	2.00	2.01	2.05	2.60*	2.03	2.84*	Tetrahedral	[4]
Sau-MntC	Mn2+	2.05	2.07	2.28	2.77*	2.32*	2.22	Tetrahedral	[5]

	Zn2+	2.05	2.10	2.56*	2.07	2.05	2.54*	Tetrahedral	[6]
MtsA	Fe	2.09	2.02	2.12	2.44*	2.79*	1.99	Tetrahedral	[7]
Syn-MntC	Mn2+	2.17	1.87	2.28	2.42*	2.86*	1.93	Tetrahedral	[8]

Table S2: Average values of RMSD, radius of gyration, SASA and intra-H bond of native **CLas-ZnuA2 native, CLas-ZnuA2** -H106C Mutant and **CLas-ZnuA2** -D247C Mutant.

S.No.	Compound	RMSD (Å)	Gyration (Å)	SASA (Å^2)	Intra-hydrogen number
1	**CLas-ZnuA2**- Native	0.96	19.38	12433.2	265.2
2	**CLas-ZnuA2**-H106C Mutant	1.25	19.31	12286.3	262.1
3	**CLas-ZnuA2**-D247C Mutant	1.38	19.48	12681.3	260.8

Table S3: Kinetic parameters of CLas-ZnuA2 interactions with different divalent metal ions: association rates, dissociation rates and equilibrium constants are given.

Analyte (divalent metal)	k_{a1} ($M^{-1}s^{-1}$)	k_{d1} (s^{-1})	K_{a2} (s^{-1})	k_{d2} (s^{-1})	K_D (M)
Ba^{2+}	8.2×10^{-5}	50.54	0.003511	0.02283	5.3×10^{-5}
Cd^{2+}	1.8×10^{8}	1571	6.6×10^{-4}	0.003931	7.1×10^{-6}
Co^{2+}	1.2×10^{4}	0.6472	0.001923	0.009397	4.3×10^{-5}
Hg^{2+}	1.1×10^{4}	0.1420	0.02584	0.01579	$4.8 \text{ X } 10^{-6}$

References

[1] N. Sharma, P. Selvakumar, S. Bhose, D.K. Ghosh, P. Kumar, A.K. Sharma, Crystal structure of a periplasmic solute binding protein in metal-free, intermediate and metal-bound states from Candidatus Liberibacter asiaticus, J. Struct. Biol. (2015).

doi:10.1016/j.jsb.2015.01.012.

[2] N. Sharma, P. Selvakumar, G. Saini, A. Warghane, D.K. Ghosh, A.K. Sharma, Crystal structure analysis in Zn 2+ -bound state and biophysical characterization of CLas-ZnuA2, Biochim. Biophys. Acta - Proteins Proteomics. 1864 (2016) 1649–1657. doi:10.1016/j.bbapap.2016.08.016.

[3] R.M. Couñago, M.P. Ween, S.L. Begg, M. Bajaj, J. Zuegg, M.L. O'Mara, M.A. Cooper, A.G. McEwan, J.C. Paton, B. Kobe, C.A. McDevitt, Imperfect coordination chemistry facilitates metal ion release in the Psa permease, Nat. Chem. Biol. (2014). doi:10.1038/nchembio.1382.

[4] M.C. Lawrence, P.A. Pilling, V.C. Epa, A.M. Berry, A.D. Ogunniyi, J.C. Paton, The crystal structure of pneumococcal surface antigen PsaA reveals a metal-binding site and a novel structure for a putative ABC-type binding protein, Structure. (1998). doi:10.1016/S0969-2126(98)00153-1.

[5] A. Gribenko, L. Mosyak, S. Ghosh, K. Parris, K. Svenson, J. Moran, L. Chu, S. Li, T. Liu, V.L. Woods, K.U. Jansen, B.A. Green, A.S. Anderson, Y. V. Matsuka, Three-dimensional structure and biophysical characterization of Staphylococcus aureus cell surface antigen-manganese transporter MntC, J. Mol. Biol. (2013). doi:10.1016/j.jmb.2013.06.033.

[6] S. Ahuja, L. Rougé, D.L. Swem, J. Sudhamsu, P. Wu, S.J. Russell, M.K. Alexander, C. Tam, M. Nishiyama, M.A. Starovasnik, C.M. Koth, Structural analysis of bacterial ABC transporter inhibition by an antibody fragment, Structure. (2015). doi:10.1016/j.str.2015.01.020.

[7] X. Sun, H.M. Baker, R. Ge, H. Sun, Q.Y. He, E.N. Baker, Crystal structure and metal binding properties of the lipoprotein MtsA, responsible for iron transport in Streptococcus

pyogenes, Biochemistry. (2009). doi:10.1021/bi900552c.

[8] V. Rukhman, R. Anati, M. Melamed-Frank, N. Adir, The MntC crystal structure suggests that import of Mn2+ in cyanobacteria is redox controlled, J. Mol. Biol. (2005). doi:10.1016/j.jmb.2005.03.006.

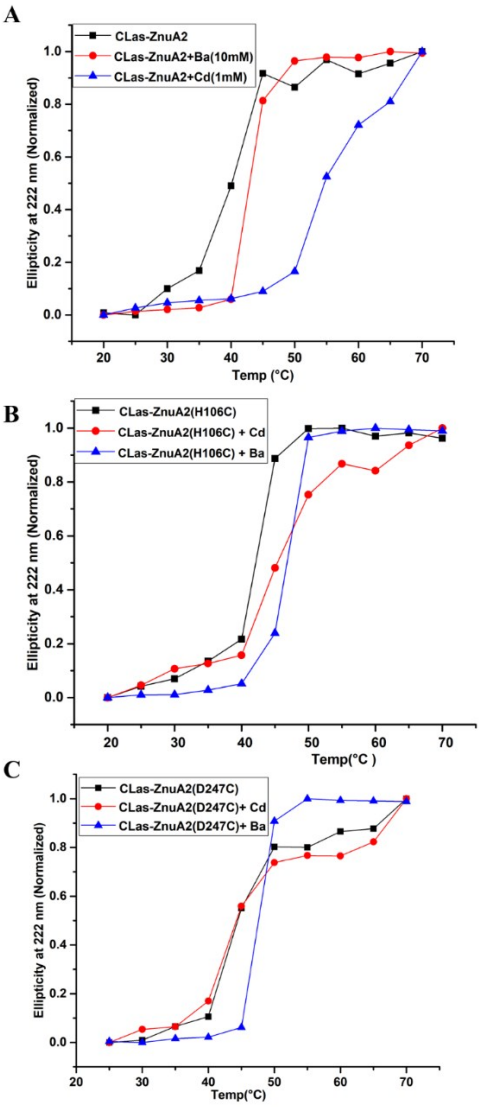

Figure S1. Circular diachroism studies (A), (B) and (C) Shows thermal denaturation of wild-type, mutant H106C and D247C monitored by CD spectroscopy at 222 nm in presence of Cd^{2+} and Ba^{2+} respectively.