Supplementary Material (ESI) for Chemical Communications

Supporting Information

Structural Probing of Zn(II), Cd(II) and Hg(II) Binding to Human Ubiquitin

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Supporting Methods

Human ubiquitin was purchased from ProtEra S.r.l. (Sesto Fiorentino, Florence, Italy). The crystallization of the four metal ion-hUb adducts was carried out by the hanging drop method at 293 K. The drops (5µl) were formed by mixing equal ratio of protein and precipitating solutions. The volume of the reservoir solution was 750 µl. The protein was dissolved in milliQ water at a concentration of 25 mg/mL. The metal ion was added to the protein solution up to a concentration close to that of precipitation.

The crystallization solution of Hg-hUb (molar ratio 1:1) was composed of 25% (w/v) PEG 1450, 50 mM HEPES pH 7.0, and 2.92 mM $Hg(CH_3COO)_2$. Orthorhombic crystals appeared within one month. A single crystal was soaked for few seconds in a cryogenic solution composed of 25% (w/v) PEG 1450K, 2.92 mM $Hg(CH_3COO)_2$ and 20% (v/v) PEG 400 and positioned in the nitrogen stream at 100 K. Diffraction data were collected at Elettra (Trieste, Italy; beam line XRD1) and recorded at a wavelength of 1.2 Å, using an oscillation range of 1°, and a crystal-to-detector distance of 100 mm.

Single crystals of Zn-hUb were grown in the presence of 25% (w/v) PEG 1450, 50 mM HEPES pH 7.0, and $Zn(CH_3COO)_2$ at a concentration ranging from 200 to 25 mM. Orthorhombic crystals formed in about two weeks at the lowest zinc concentration, while cubic crystals formed in a week at the highest concentration. At intermediate concentrations around 75 mM both crystalline forms precipitated. Prior to data collection crystals were briefly transferred into a cryo-protectant solution (25% (w/v) PEG 1450, 200 mM or 25 mM $Zn(CH_3COO)_2$, 15% (v/v) PEG 400). Data from Zn-hUb cubic and orthorhombic crystals were collected at Elettra (Trieste, Italy; beam line XRD1), at a wavelength of 1.0 Å, using an oscillation range of 1°, and a crystal-to-detector distance of 130 mm.

Cd-hUb was crystallized using a precipitating solution composed of 25% (w/v) PEG 1450, 50 mM HEPES pH 7.0 and 200 mM $Cd(CH_3COO)_2$. Cubic crystals grew within two weeks.

Cd-hUb data were collected from single crystals at 100 K at beam line ID21, ESFR (Grenoble, France) using a wavelength of 0.997 Å, an oscillation range of 1°, and a crystal-to-detector distance of 150 mm.

All data were processed and scaled using DENZO/SCALEPACK package[1] and the statistics are reported in Table 1SI. The structures were solved by molecular replacement, using the structure of native ubiquitin (1UBQ) as probe. The metal ions were added in the regions with high electronic density (contoured more than 5σ on the ($2F_o$ - F_c) map) in the electron density maps, having a distance from the protein donor atom(s) shorter than the sum of the Van der Waals radii and a correct geometry. The occupancy factors for the metal ions incorporated into the models were refined starting with thermal factors (B) of the same magnitude as those of the protein atoms. The refinements were performed with CNS[2] with a starting σ cut-off on amplitudes of 2.0, which was later decreased to 0. A 5% of the total data was randomly selected for R_{free} calculations. The models were rebuilt with the graphic program XtalView[3]. In the final stage of the refinement of Hg-hUb and Zn-hUb the solvent network was built. Water molecules were automatically added, and after a visual inspection they were conserved in the model only if contoured at 0.8σ on the ($2F_o$ - F_c) map and if they fell into an appropriate hydrogen bonding environment. Only few water molecules were manually added to the Cd-hUb structure. The high thermal factors of this structure (Table 2S1) with respect to those of Zn-hUb and Hg-hUb are probably due to the low data resolution and to an intrinsic crystal disorder enhanced by the high crystal symmetry. All the refinement statistics are listed in Table 2SI.

The Ramachandran plots of the solved structures, obtained using PROCHECK[4], show the majority of the residues lying in the most favored and allowed regions, and the remaining in the additional allowed regions. The C-terminal region of the protein showed a high degree of disorder, only the amino acids clearly visible in the electron density maps were included in the structure and refined.

The cubic crystals of Zn-hUb diffracted at the maximum resolution of 3.6 Å with cell parameters $a = b = c$= 105.143 Å. Data collection statistics gave R_{sym} = 0.154 and mosaicity equal to 1. These data did not allow a resolution of the structure with the required accuracy.

References

1 Z. Otwinowski, W. Minor, *Methods Enzymol.* 1997**, 276,** 307-326.

2 A. T. Brunger, P. D. Adams, G. M. Clore, W. L. DeLano, P. Gros, R. W. Grosse-Kunstleve, J. S. Jiang, J. Kuszewski, M. Nilges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonson, G. L. Warren, *Acta Crystallogr .D. Biol .Crystallogr.* 1998**, 54,** 905-921.

3 D. E. McRee, *J. Struct. Biol.* 1999**, 125,**156-165.

4 R. A. Laskowski, M. W. MacArthur, D. S. Moss, J. M. Thornton, *J. Appl. Crystallogr.* 1993**, 26,** 283-291.

Supporting Tables

Table 1SI. Cell parameters and data collection statistics for ubiquitin crystallized in the presence of metal ions.

	Zn-hUb	**Cd-hUb**	**Hg-hUb**
Space group	$P2_12_12_1$	$P4_332$	$P2_12_12_1$
Unit cell *a*, *b* ,*c*, (Å)	44.36, 50.95, 93.32	105.253	28.02, 42.83, 50.37
N° molecules in a.u.	3	2	1
Resolution range*	32.5-1.8(1.84-1.80)	74.5-3.0 (3.1-3.0)	24.5-1.8 (1.85-1.80)
Measured reflections	70229	348 734	29 818
Unique reflections	19140	4357	5484
Completeness (%)*	94.8	100 (100)	93.7 (58.8)
Redundancy	3.7	80	5.4
R_{sym}*	0.114(0.513)	0.078 (0.70)	0.074 (0.162)
$I/\sigma(I)$*	11.7(1.3)	76.5 (10.4)	32.5 (15.0)

*Value in parenthesis refers to the last resolution shell

Table 2SI. Refinement statistics for ubiquitin crystallized in the presence of metal ions.

	Zn-hUb	**Cd-hUb**	**Hg-hUb**
No. protein atoms	1706	1166	575
No. metal atoms	1	12	1
No. water molecules	127	7	105
R (%)	22.2	27.9	23.5
R_{free} (%)	27.1	30.1	29.3
B mean ($Å^2$)	25.8	74.1	20.9
B (Wilson Plot, $Å^2$)	17.2	73.8	20.1
B protein atoms ($Å^2$)	24.0	74.3	19.3
B metal atoms ($Å^2$)	44.1	64.2	28.4
B solvent atoms ($Å^2$)	45.4	58.7	29.8
rmsd bond length (Å)	0.005	0.012	0.005
rmsd bond angles (°)	1.2	1.9	1.2

Table 3SI. Metal ion – protein donor atom distances below 3.5 Å in the most relevant metal binding sites of hUb.

Me-hUb adduct	**Metal ion sites**	**Chain A**			**Chain B**		
		Protein residues		Dist. (Å)	Protein residues		Dist. (Å)
Hg-hUb	Hg1	His68	NE2	2.2			
		Gln31*	NE2	2.4			
		Gln31*	OE1	2.7			
Zn-hUb	Zn1	His68	NE2	2.5	His68	NE2	2.4
		W1	O	3.2			
		W2	O	2.8			
Cd-hUb	Cd1	Glu64	OE1	2.3	Glu64*	OE1	2.6
		Glu64	OE2	2.5	Glu64*	OE2	2.3
		His68*	NE2	2.5	His68	NE2	2.3
		W1		2.6			
		W2		2.5			
	Cd2	Glu16	OE1	2.5	Glu16	OE1	2.5
		Glu16	OE2	2.3	Glu16	OE2	2.4
		Asp32*	OD1	2.6	Asp32*	OD2	2.6
		Asp32*	OD2	2.7			
		Met1	NH	2.7	Met1	NH	2.8
		Met1	CO	2.5	Met1	CO	2.6
		W3		2.4	W5		2.3
	Cd3	Glu18	OE1	2.7	Glu18*	OE1	2.8
		Glu18	OE2	2.3	Glu18*	OE2	2.4
		Asp21*	OD1	2.6	Asp21	OD1	2.6
		Asp21*	OD2	2.6	Asp21	OD2	2.5
		Glu18*	OE1	2.7	W6		2.9
		W4		3.4	W7		2.5

* indicates that the residue belongs to a symmetric molecule

Table 4SI. Occupancy and thermal factors of Hg(II), Zn(II), and Cd(II) ions in the binding sites of Me-hUb adducts.

Me-hUb adduct	**Metal ion sites**	**Chain A**		**Chain B**	
		occupancy factor*	thermal factor (B)	occupancy factor*	thermal factor (B)
Hg-hUb	Hg1	0.77	28.4		
Zn-hUb	Zn1	0.50	44.1		
Cd-hUb	Cd1	0.70	67.1	0.20	63.9
	Cd2	0.85	59.4	0.45	64.5
	Cd3	0.85	58.4	0.75	61.2

* Occupancy factor defines the fraction of crystallographic sites occupied by metal ions.

Supporting Figure

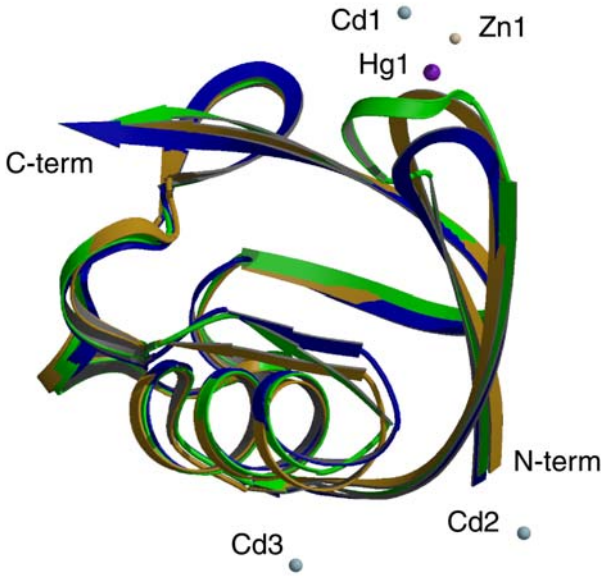

Figure 1SI. Superposition of the three Me-hUb adduct structures. Only the Cα trace of the protein molecules is shown in different colours. Zn-hUb is shown in green, Cd-hUb is shown in blue and Hg-hUb is shown in gold. Metal ions are shown as spheres of different colours: brown indicates Zn(II), light blue indicates Cd(II) and violet indicates Hg(II). The N-terminal (N-term) and C-terminal (C-term) regions of the protein are also indicated.