Electronic Supplementary Information

Selective interaction of Co^{2+}–Ca^{2+}–concanavalin A with high mannose *N*-glycans

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Supplementary materials and methods

1. Crystallization, data collection and structure determination of Co^{2+}–Ca^{2+}–Concanavalin A

The initial crystallization conditions were tested with crystallization kits, such as Crystal Screen 1™ and 2™ (Hampton Research, California, USA) in 24-well Crystallization Plates (Hampton Research, California, USA). Concanavalin A (ConA) isolated from *Canavalia ensiformis* (Jack bean) was purchased from Sigma (C2010; St. Louis, MO, USA). A total of 2.0 μL mixture of the *Canavalia ensiformis* (Jack bean) Con A (2 mg/mL) in buffer (20 mM Tris, 50 mM NaCl, pH 7.4) and an equal volume of crystallization solution were equilibrated against 800 μL reservoir solution at 22 °C. Crystals were obtained in the #25 condition (0.01 M cobalt(II) chloride hexahydrate, 0.1 M MES monohydrate pH 6.5, 1.8 M ammonium sulfate) using Crystal Screen 2™ crystallization kit. For the native data collection, a single crystal was cryoprotected with the reservoir solution supplemented with 30% sucrose and flash-frozen directly in a –173°C nitrogen stream using a nylon loop. A dataset consisting of 360 frames with 1° oscillation at a wavelength of 1.0 was collected using an DECTRIS EIGER X 9M detector at beamline 5C of the Pohang Light Source II (PLS II) at the Pohang Accelerator Laboratory (PAL, Pohang, Korea).

Data reduction and scaling were processed using X-ray Detector Software (XDS).[1] Molecular replacement computations with Mn^{2+}–Ca^{2+}–ConA (PDB accession 5YGM) were performed using the program Phaser.[2] Model building and refinement were accomplished using Coot and PHENIX.refine.[3, 4] Manual model building of missing parts and subsequent refinement were performed several times with Coot4 and Refmac5, respectively, using native data at a resolution of 2.83 Å.[5] The refinement statistics are presented in Table S1. The atomic coordinates and structure factors were deposited in the Protein Data Bank using the accession code 8I7Q (www.rcsb.org/pdb). The final refined model contains Co^{2+}–Ca^{2+}–ConA with R_{work} and R_{free} values of 0.258 and 0.297, respectively.

Table S1. Data collection and refinement statistics for Co(II)-Ca(II)-Concanavalin A

Statistics	Co(II)-Ca(II)-Concanavalin A
Data collection	
Space group	I 2 2 2
Cell dimensions (Å)	
a, b, c (Å)	61.82, 86.05, 88.6
α, β, γ (°)	90, 90, 90
Resolution (Å)	27.93 – 2.83 (2.93 – 2.83) *
R_{merge} [a]	0.276 (1.573)
$I / \sigma I$	7.7 (2.1)
$CC_{1/2}$ [b]	0.996 (0.908)
Reflections, total / unique	76423 / 5904
Completeness (%)	99.9 (100.0)
Redundancy	12.9 (13.7)
Structure refinement	
Resolution (Å)	27.93 - 2.83
Reflections	5,882
R_{work} [c] / R_{free}	0.258 / 0.297
Wilson B factor (Å^2)	58.68
No. atoms, protein / water	1,807 / 19
R.m.s.deviation	
Bond lengths (Å)	0.005
Angles (°)	0.93
Rotamer outliers (%)	0.99
Average B-factor (Å^2)	63.88
Protein	63.87
Ligands	60.74
Solvent	65.13
Ramachandran plot (%)	
Favored region	97.02
Allowed region	2.98

*Values in parentheses are for highest-resolution shell.

[a] $R_{merge} = \Sigma |I_{obs} - I_{avg}| / I_{obs}$, where I_{obs} is the observed intensity of individual reflection and I_{avg} is the average over symmetry equivalents

[b] $CC_{1/2}$ = Pearson correlation coefficient between two random half datasets.

[c] $R_{work} = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively. The R_{free} was calculated using 5% of the data.

2. Measurements of fluorescence anisotropy between *N*-glycans-2AB and concanavalin A

2-Aminobenzamide (AB)-labeled oligomannose oligosaccharide with 9 mannosyl residues (Ludger, 2AB-labeled Man_9 glycan, CAB-MAN9-01), 2AB-labeled asialo, galactosylated triantennary oligosaccharide (Ludger, 2AB-labeled NA_3 glycan, CAB-NA3-01), 2AB-labeled asialo, agalactosylated triantennary oligosaccharide (Ludger, 2AB-labeled NGA_3 glycan, CAB-NGA3-01) and 2AB-labeled sialylated triantennary oligosaccharide (Ludger, 2AB-labeled A_3 glycan, CAB-A3-01) were purchased for the fluorescence anisotropy (FA) experiment.[6] Man_9-2AB has α-D-mannose as its terminal carbohydrates. NA_3-2AB has β-D-galactose as its terminal carbohydrates. NGA_3-2AB has β-N-acetyl-D-glucosamine as its terminal carbohydrates. A_3-2AB has α-N-acetyl-D-neuraminic acid as its terminal carbohydrates.

N-glycans-2AB binding to ConA was observed using the FA. The FA was measured by FP8300 (JASCO, Japan) using cuvettes (JASCO, J/3 type, material Q [10 x 10 mm]). The excitation and emission of *N*-glycans-2AB were measured at 337 and 433 nm.[6] All experiments were measured at parameters including bandwidth: 5 nm, response: 1.0 sec, and PMT voltage: 800 V, at 25 °C. The 2AB attached 20 nM *N*-glycans in 800 μL of buffer (20 mM Tris-HCl, 50 mM NaCl at pH 7.4) was titrated by adding ConA, and anisotropy was measured three times after 2 min reaction. The dissociation constant between ConA and *N*-glycans-2AB was obtained using the 1:1 binding model. The overall calculation was measured using the following equations.[6, 7]

$$F_{bound} = \frac{r - r_{free}}{r_{bound} - r_{free}} \qquad \text{Eq. 1}$$

and $P + D \rightleftharpoons PD$

$$K_d = \frac{[P][D]}{[PD]}$$

$$F_{bound} = \frac{P_{total} + D_{total} + K_d - \sqrt{(P_{total} + D_{total} + K_d)^2 - 4P_{total}D_{total}}}{2D_{total}} \quad \text{Eq. 2}$$

where, F_{bound} (fraction bound) is Fraction of Bound ConA to *N*-glycans-2AB, r_{free} is anisotropy of unbound *N*-glycans-2AB, r_{bound} is anisotropy value of ConA-*N*-glycans-2AB bound when saturated, P is the concentration of ConA, D is the concentration of *N*-glycans-2AB, and K_d is dissociation constant.

3. Preparation of Co^{2+}–Ca^{2+}–concanavalin A

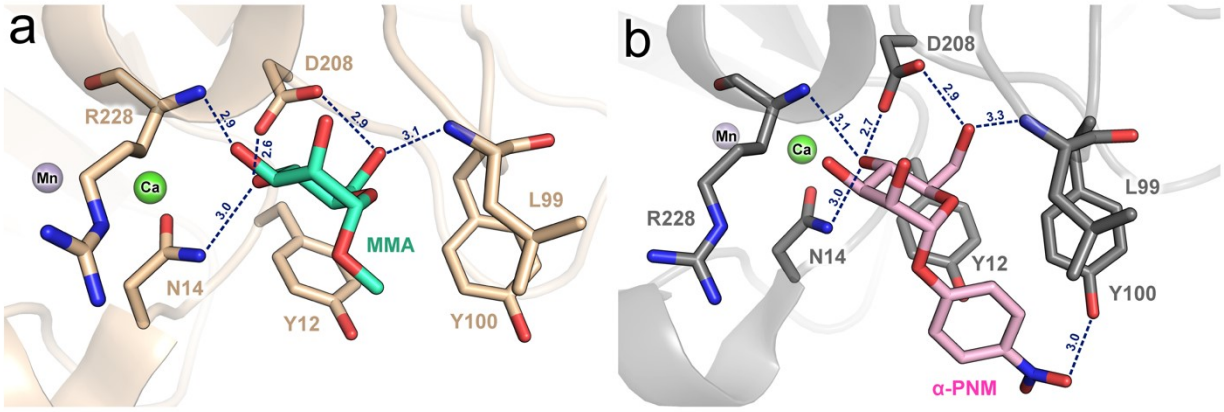
Co^{2+}–Ca^{2+}–Concanavalin A was prepared by adding 10.0 eq cobalt(II) chloride hexahydrate (Sigma-Aldrich) to Concanavalin A in a buffer solution (20 mM Tris, 50 mM NaCl, 1 mM DTT and pH 7.4) at 4 °C for 1 h to exchange its coordination with manganese ions and cobalt ions. The mixture was filtered using a 10 kDa cut-off membrane filter (Merck Millipore) at 4 °C and centrifuged at 2,095 × *g* to eliminate metal ions not bound to concanavalin A.[8] The mixture was washed at least five times with exchange buffer (20 mM Tris, 50 mM NaCl, 1 mM DTT and pH 7.4) to obtain Co^{2+}–Ca^{2+}–concanavalin A.

4. Preparation of apo-concanavalin A

Apo-ConA was prepared by adding 3 M HCl to Mn^{2+}–Ca^{2+}–ConA at 4 °C for 2 h to eliminate its coordination with metal ions. The mixture was filtered using a 10 kDa cut-off membrane filter to eliminate metal ions. Apo-ConA was then washed at least five times with exchange buffer (20 mM Tris-HCl and 50 mM NaCl, 1 mM DTT; pH 7.4)

Supplementary Figures

Fig. S1 Carbohydrate-binding domain (CBD) of concanavalin A (ConA) in complex with *N*-glycan. Structures of the complex of native ConA with (a) methyl α-D-mannopyranoside (MMA, green-cyan, PDB: 5CNA) and (b) 4′-nitrophenyl-α-D-mannopyranoside (α-PNM, pink, PDB: 1VAM) are shown. Purple and green spheres represent Mn^{2+} and Ca^{2+} ions, respectively. Unit: Å



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