Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information

Influence of high-mannose glycan whose glucose moiety is substituted with 5-thioglucose on calnexin/calreticulin cycle.

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Materials and methods

Materials

M9-BODIPYwas synthesized following our previous report[1]. Deoxynojirimycin and deoxymannojirimycin were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). UDP-glucose disodium salt, calcium chloride, Triton X-100, Tris hydroxymethyl aminomethane, hydrochloride and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). UDP-5-thioglucose was synthesized from 5-thioglucose (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) following previous report[2].

Preparation of ER quality control-related proteins

Drosophila UGGT was a kind from Dr. Karin Reinisch and Dr. Stephanie Hamill (Department of Cell Biology, Yale University School of Medicine), which was tagged with a C-terminal $(His)_6$ tag and expressed in Hi-5 insect cells. Recombinant human CNX was produced by E. coli. A cDNA encoding 19-481 amino acids of human CNX was cloned into pCold I expression plasmid (Takara Bio., Ohtsu, Japan), which was designed to produce N-terminally $(His)_6$-tagged proteins. Similarly, an expression plasmid encoding 18-469 amino acids of human CMG was constructed. Human CRT (23-417 aa) was cloned into pCold Ibetween *EcoRI* and *XbaI* sites. Mutagenesis of the expression plasmid encoding for CRT was performed using with QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) according to the manufacturer's instructions. The obtained plasmid was transfected with BL21 cells, and the recombinant protein was expressed and purified using with Ni-NTA agarose (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. The obtained proteins

were dialyzed in 10 mM MOPS buffer (pH 7.5) using Slide-A-Lyzer dialysis cassettes (10000 MWCO, PIERCE, IL, USA). The yields of CNX and CMG were 1.1 and 0.8 mg from 1 L culture, respectively. GII was prepared following previous report[3].

Glucose transfer reaction by UGGT

The reaction mixture containing 10 nM acceptor substrate M9-BODIPY, 1 mM UDP-glucose, 0.6 μM UGGT, 250 μM deoxynojirimycin, 250 μM deoxymannojirimycin, 10 mM $CaCl_2$ and 4 mM Tris-HCl (pH 8.0), was prepared, and then the solution was incubated for 10 minutes at 37 °C. After incubation, 20 μL of the solution was removed by a micropipet and diluted with 20 μL of CH_3CN to stop the enzymatic reaction. The glucose transfer yield was analyzed by HPLC.

Measurement of BODIPY-conjugated glycan by HPLC and MS.

The glycan-BODIPY sample was analyzed by HPLC under the following conditions: Inertsil Amide column (4.6 mmϕ × 150 mm), mobile phase CH_3CN/100 mM ammonium formate (pH 4.5), linear gradient from 65:35 to 55:45 in 20 min, flow rate 1.0 ml/min at 40 °C. BODIPY-labeled glycans were quantified by fluorescence intensities (λex = 488 nm, λem = 520 nm) with Waters 2475 fluorescence detector. MALDI-TOF mass spectra were recorded on a Bruker Daltonics autoflex speed with 2,5-dihydroxybenzoic acid as the matrix.

Analysis of lectin chaperone-glycan interaction by ultrafiltration method

The ultrafiltration method for binding specificity analysis is well-described in our recent report[4]. Briefly, molecular chaperone and glycan were mixed in a buffer (10 mM

MOPS (pH 7.5), 10 mM $CaCl_2$), and then the solution was equilibrated at room temperature. After 5 min, the solution was ultrafiltrated with VIVACON500 according to manufacturer's instructions at 5000 g for 2 min. The filtrate was analyzed by HPLC.

Alignment of amino acid sequences and rendering of chaperone structure.

The alignment program Clustalx was used to align sequences of human CRT, in comparison with mouse CRT. All sequences in FASTA format were obtained from Genbank database. Their accession numbers were the following: mouse CRT (NP_031617.1) and human CRT (NP_004334.1). PDB file using for rendering 3D models of mouse and human CRT were 3O0V and 3POW, respectively. Predicted 3D models of human CNX and human calmegin were referred to our previous report[5]. All the analysis and molecular representations were rendered in PyMOL (Schrödinger).

Glucose hydrolysis reaction by glucosidase-II

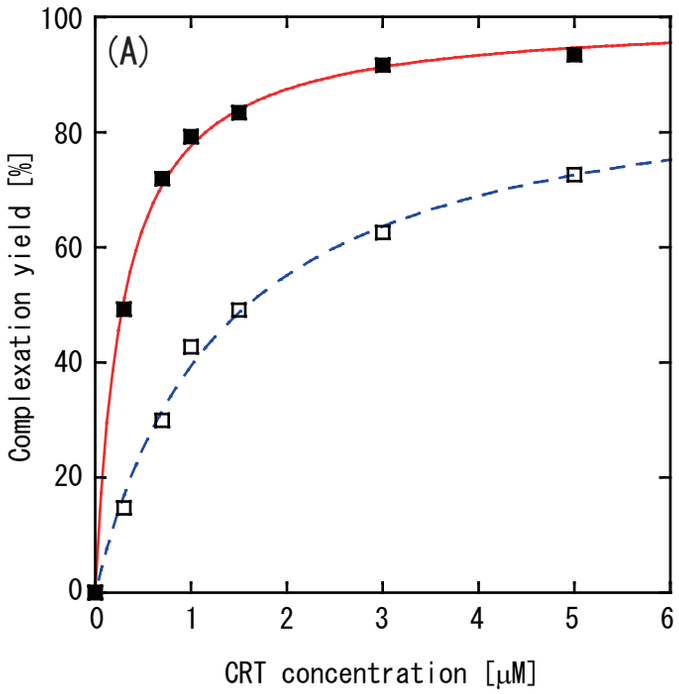
The reaction mixture containing 20 nM G1M9-BODIPY, 1 mM $CaCl_2$ and 1 μM glucosidase II-immobilized beads in 10 mM HEPES (pH 7.5) was prepared, and then the solution was incubated for 10 minutes at 37 °C. The reaction was stopped by adding CH_3CN. The glucose hydrolysis yield was analyzed by HPLC.

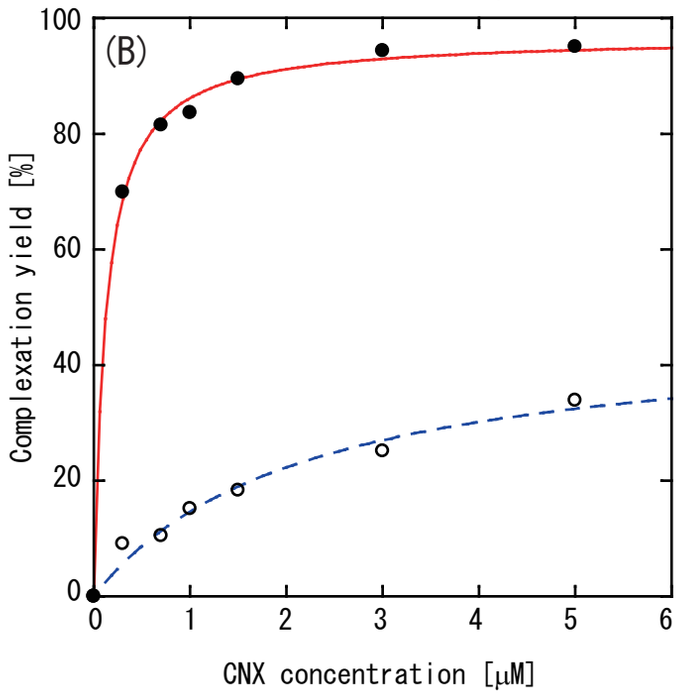
Supplementary figures

Figure legends

Figure S1. Influence of 5-thioglucose on interaction between lectin chaperone and synthetic N-glycan derivatives. The reaction mixture contained 20 nM G1M9-BODIPY or 5S-G1M9-BODIPY and appropriate concentration of lectin chaperone. *Filled symbol*, G1M9-BODIPY; *open symbol*, 5S-G1M9-BODIPY. (A) Calreticulin. (B)Calnexin. (C)Calmegin.

Figure S2. Glucose recognition site of human calreticulin on predicted structure of human calnexin or human calmegin. Body color *blue*, human calreticulin; *green*, human calnexin; *red*, human calmegin.





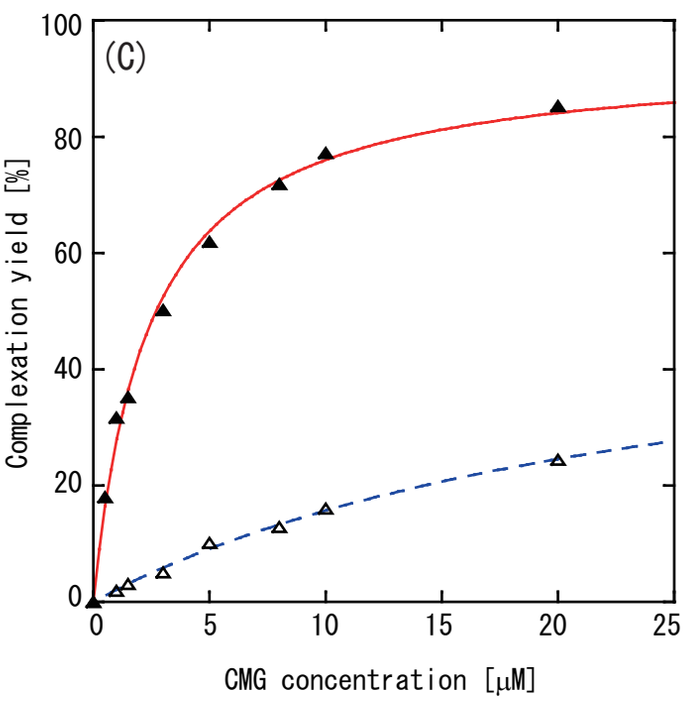


Figure S1
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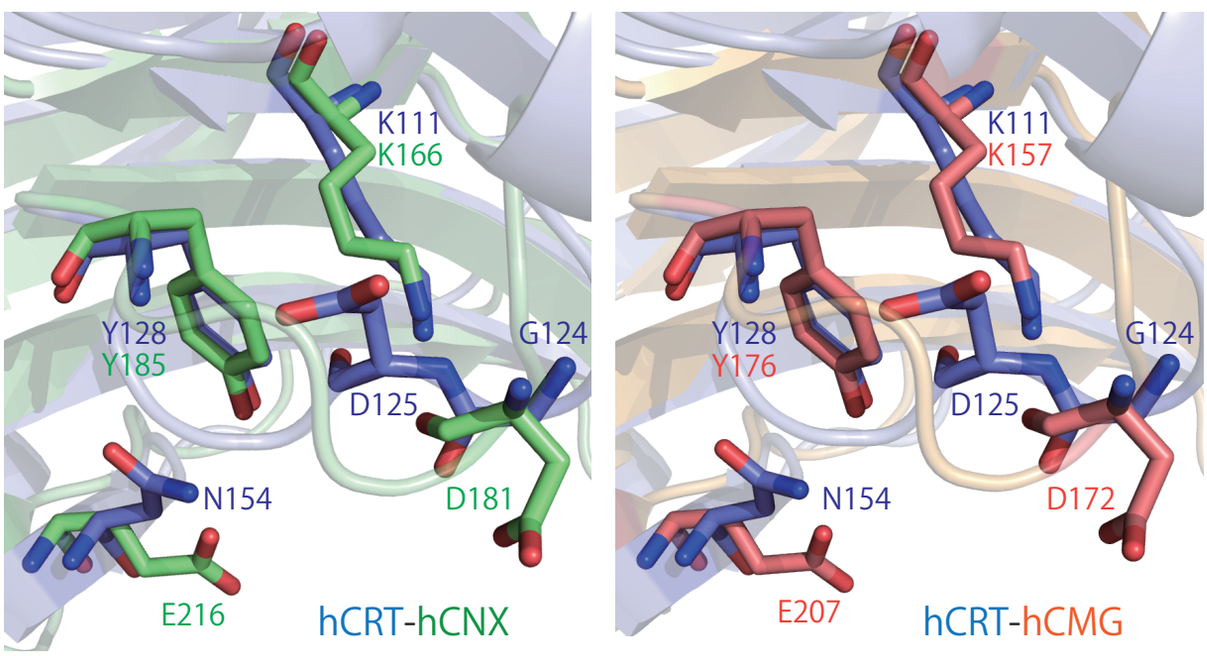

Figure S2

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Table S1. Ratio of comlexation yield between G1M9 and 5S-G1M9.

CRT	G1M9/5S-G1M9
Wild type	1.7
D125N	1.5
D125G	1.3
D125S	1.5
N154D	2.6
N154G	2.5
N154S	2.1

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