Crystallization and preliminary X-ray diffraction analysis of Gos1p, a yeast SNARE protein

Baoyun Cheng,^{a,b} Yujie Zhang,^{a,b}
Gongrui Guo^{a,b} and Yongxiang
Gao^{a,b*}

^aSchool of Life Sciences, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui 230026, People's Republic of China, and ^bKey Laboratory of Structural Biology, Chinese Academy of Sciences, 96 Jinzhai Road, Hefei, Anhui 230026, People's Republic of China

Correspondence e-mail:
yxxgao@mail.ustc.edu.cn

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The Gos1 protein (Golgi SNAP receptor complex member 1) is involved in the SNARE complex, which is the core machinery that drives membrane fusion between cargo-carrying vesicles and their target membranes in the secretory and endocytic pathways in yeast. Truncated versions of the Gos1 protein from *Saccharomyces cerevisiae* were cloned, expressed, purified and crystallized. The crystal belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 39.67$, $b = 43.58$, $c = 81.94$ Å, $\alpha = \beta = \gamma = 90^\circ$. An X-ray diffraction data set was collected at 100 K to 1.63 Å resolution. Matthews coefficient (V_M) calculations suggest that one molecule is present in the asymmetric unit, corresponding to a solvent content of ~55%.

1. Introduction

Saccharomyces cerevisiae Gos1 protein (Golgi SNAP receptor complex member 1) is a member of the SNARE protein family, which includes integral membrane proteins involved in membrane fusion. SNAREs were originally divided into v-SNAREs and t-SNAREs according to their vesicle or target membrane localization (Chen & Scheller, 2001). In the secretory and endocytic pathways of yeast, the SNARE proteins are critical for membrane fusion. SNARE proteins are typically C-terminally anchored and have the potential to associate with each other, forming parallel bundles of amphipathic helices (the SNARE core complex; Pelham, 1999). These SNARE complexes mediate membrane fusion with other regulatory proteins, such as the Sec1/Munc18 (SM) family of proteins (Latham *et al.*, 2007).

The *S. cerevisiae* SNARE Gos1p is an integral membrane protein with a C-terminal membrane anchor and a SNARE motif with a strong propensity to form coiled coils. Gos1p (v-SNARE), likely complexed with a t-SNARE, acts in the endoplasmic reticulum (ER)–Golgi and/or during intra-Golgi transport (McNew *et al.*, 1998). It is a 223-amino-acid protein with a predicted molecular mass of 25 394 Da encoded by the ORF YHL031c on chromosome VIII (McNew *et al.*, 1997). Analysis of the Gos1p primary sequence indicates a modest similarity to the mammalian Golgi SNARE GOS-28/GS28, with 27.6% sequence identity (McNew *et al.*, 1998). GS28 has been implicated in vesicular transport between intra-Golgi networks and between endoplasmic reticulum (ER) and Golgi, which may play a protective role against cytotoxicity induced by hydrogen peroxide under glutathione-depleted conditions in neuronal cells; and participates in the docking and fusion stages of ER to *cis*-Golgi transport (Lee *et al.*, 2011).

The present evidence indicates that Gos1p and other SNARE proteins form complexes involved in the fusion of vesicles with their target membranes. The structural information demonstrates that v-SNAREs (anchored in the vesicle membrane) zipper into a coiled-coil four-helix bundle with cognate t-SNAREs (anchored in the target membrane) (Krishnakumar *et al.*, 2011). In neuronal synapses, the structure of the synaptic fusion complex (syntaxin-1A, synaptobrevin-II, N- and C-terminal fragments of SNAP-25B) is a four-helix bundle that may also be involved in promoting membrane fusion by a

conformational change and subsequent antiparallel association of α -helices with the assistance of regulatory proteins (Sutton *et al.*, 1998). In yeast, Gos1p interacts with Sed5p, Ykt6 and Sft1, playing a central role in vesicle-target recognition and regulating membrane fusion (Tsui & Banfield, 2000; Parlati *et al.*, 2002). Gos1p SNARE complexes are most likely to play a vital regulatory role in the mechanism of membrane fusion, similar to synaptic fusion complexes.

According to structural and functional predictions, the C-terminal region of Gos1p includes a transmembrane domain and a conserved SNARE motif involved in the formation of the core complex, while the significance of the N-terminal region is less clear (Dulubova *et al.*, 2001). In the process of vesicle trafficking and membrane fusion, the N-terminal domain is important for binding to other proteins, such as neuronal Sec1, that modulate syntaxin function (Munson *et al.*, 2000). In some syntaxins, the N-terminal domain binds to the SNARE motif, and this intramolecular interaction decreases the rate of association with the partner SNAREs, such as syntaxin 1a and Sso1p (Misura *et al.*, 2002). The three-dimensional structure of Gos1p may provide insight into the mechanism of SNARE assembly and its intramolecular and intermolecular regulation. We attempted to express full-length Gos1p; unfortunately, we encountered the problem of protein degradation during the purification process. We therefore made a soluble stable truncation of Gos1p (residues 1–115), removing the C-terminal domain. Here, we report the expression and crystallization of the N-terminal domain of *S. cerevisiae* Gos1p (residues 1–115) and provide preliminary data of its X-ray analysis.

2. Materials and methods

2.1. Cloning, expression and purification of yeast Gos1p

The gene encoding yeast Gos1 (residues 1–115) was amplified by PCR from the *S. cerevisiae* genome using primers containing *Nde*I and *Xho*I restriction sites. The sense primer was 5'-GGA ATT CCA TAT GAT GAG CTC ACA ACC GTC TTT CG-3' and the antisense primer was 5'-CCG CTC GAG TTA GCT GAA TAA CAA GTT TAG TCT GTT GCG-3'. The PCR products were digested by the restriction endonucleases *Nde*I/*Xho*I and inserted into the *Nde*I/*Xho*I site of the pET-28a vector (Novagen) to encode a recombinant

protein with an N-terminal hexahistidine tag. The ligation mixture was transformed into chemically competent *Escherichia coli* TOP10 cells, and the insertion was verified by PCR and DNA sequencing. The recombinant plasmid was then transformed into *E. coli* BL21 (DE3) cells for protein expression. Cultures of bacteria carrying pET-28a-Gos1 were pre-cultured in 10 ml Luria-Bertani (LB) medium containing kanamycin ($50 \mu\text{g ml}^{-1}$) at 310 K overnight. The cells were transferred to 4 l of LB medium and were cultured at 310 K until the OD_{600} reached ~ 0.8 , at which point they were induced using 0.25 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) at 289 K. The *E. coli* cells were harvested 20 h later by centrifugation, resuspended in lysis buffer A (20 mM Tris-HCl pH 8.0, 100 mM NaCl) and disrupted by sonication. The cell lysate was centrifuged at 12 000g for 30 min at 277 K. The recombinant Gos1 protein in the supernatant was loaded onto an Ni^{2+} -NTA column (GE Healthcare, USA) previously equilibrated with lysis buffer A. The recombinant protein was eluted from the column with 30 ml elution buffer B (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 300 mM imidazole). The eluted protein was subjected to size-exclusion chromatography using a HiLoad 16/60 Superdex 75 column (GE Healthcare, USA) equilibrated with lysis buffer A on an ÄKTAprime plus system (GE Healthcare). The concentration of the final purified recombinant protein was determined to be approximately 6 mg ml^{-1} in buffer A using the BCA Protein Assay Kit (Pierce, USA), and its purity was assessed by SDS-PAGE, after which it was stored at 193 K until use.

2.2. Crystallization

Crystals of Gos1 protein were grown using the hanging-drop vapour-diffusion method at 289 K. Initial screening of the crystallization conditions was performed using the Proplex screen (Molecular Dimensions) in 48-well plates. The crystallization drop was prepared using $1.0 \mu\text{l}$ protein solution (6 mg ml^{-1}) and an equal volume of crystallization reservoir solution, and was equilibrated against $150 \mu\text{l}$ precipitant solution. A series of cuboid-shaped crystals of different sizes were obtained. Diffraction-quality crystals appeared after 4 d in condition No. 44 of Box 1 of the Proplex screen (15% PEG 6000, 0.1 M magnesium acetate, 0.1 M sodium cacodylate pH 6.5) and grew to full size within a week.

Figure 1
15% SDS-PAGE of the purified *S. cerevisiae* Gos1 protein (residues 1–115) stained using Coomassie Brilliant Blue. Right lane, purified Gos1 protein from the Ni-NTA column ($\sim 13 \text{ kDa}$); left lane, molecular-weight marker (labelled in kDa).

Figure 2
Crystals of *S. cerevisiae* Gos1 protein (residues 1–115) were grown under the condition 15% PEG 6000, 0.1 M magnesium acetate, 0.1 M sodium cacodylate pH 6.5 by the hanging-drop method.

Figure 3

An X-ray diffraction pattern of a native crystal of Gos1 protein recorded using an MX225 CCD detector.

2.3. X-ray data collection and processing

X-ray diffraction data were collected using a Jupiter CCD detector at 100 K on beamline BL-17U of the Shanghai Synchrotron Radiation Facility (SSRF), People's Republic of China. The crystals were transferred into a cryoprotectant consisting of 25%(v/v) glycerol, mounted on a loop and then flash-cooled in liquid nitrogen. For the X-ray data analysis, 180 diffraction images were collected with an oscillation angle of 1.0° using X-rays with a wavelength of 0.97915 Å and 0.8 s exposures. The crystal-to-detector distance was set to 170 mm. The data sets were scaled and merged using the *HKL-2000* program package (Otwinowski & Minor, 1997).

3. Results and discussion

The yeast Gos1 (residues 1–115) protein with a His₆ tag at the N-terminus was expressed in *E. coli* and purified. SDS-PAGE of the purified Gos1p showed a single band with a molecular mass of approximately 13 kDa (Fig. 1). Good-quality crystals were obtained using a precipitant consisting of 15% PEG 6000, 0.1 M magnesium acetate, 0.1 M sodium cacodylate pH 6.5. The cuboid-shaped crystals grew to $0.1 \times 0.12 \times 0.1$ mm after 4 d (Fig. 2). A total of 180 diffraction images were collected to 1.63 Å resolution on beamline BL-17U at the SSRF (Fig. 3). The Gos1p crystal belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 39.67$, $b = 43.58$, $c = 81.94$ Å, $\alpha = \beta = \gamma = 90^\circ$. The overall R_{merge} was 8.2% and the R_{merge} in the outermost shell was 30.9%. The Matthews coefficient of $2.74 \text{ Å}^3 \text{ Da}^{-1}$ suggested that there was one Gos1 protein molecule in the asymmetric unit (Matthews, 1968). The statistics of the collected data are summarized in Table 1. Unfortunately, we failed to solve the

Table 1

Summary of data-collection statistics.

Values in parentheses are for the outermost resolution shell.

Space group	$P2_12_12_1$
Wavelength (Å)	0.97915
Unit-cell parameters (Å, °)	$a = 39.669$, $b = 43.58$, $c = 81.94$, $\alpha = \beta = \gamma = 90.0$
Resolution (Å)	50.0–1.63
No. of observed reflections	135729
No. of unique reflections	35928
Completeness (%)	99.7 (99.6)
Multiplicity	3.8 (3.7)
Average $I/\sigma(I)$	11.39 (4.11)
$R_{\text{merge}}^\dagger$ (%)	8.2 (30.9)
No. of subunits per asymmetric unit	1
Solvent content (%)	55.09

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection hkl and $\langle I(hkl) \rangle$ is the mean intensity of reflection hkl .

crystal structure by molecular replacement using the structure of the tellurite-resistance protein COG3793 from *Nostoc punctiforme* as a search model (PDB entry 2ou3; Joint Center for Structural Genomics, unpublished work; 25% sequence identity). Multi-wavelength anomalous diffraction by soaking in heavy-atom solution is in progress as a phase-solution strategy.

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References

- Chen, Y. A. & Scheller, R. H. (2001). *Nature Rev. Mol. Cell Biol.* **2**, 98–106.
- Dulubova, I., Yamaguchi, T., Wang, Y., Südhof, T. C. & Rizo, J. (2001). *Nature Struct. Biol.* **8**, 258–264.
- Krishnakumar, S. S., Radoff, D. T., Kümmel, D., Giraudo, C. G., Li, F., Khandan, L., Baguley, S. W., Coleman, J., Reinisch, K. M., Pincet, F. & Rothman, J. E. (2011). *Nature Struct. Mol. Biol.* **18**, 934–940.
- Latham, C. F., Hu, S.-H., Gee, C. L., Armishaw, C. J., Alewood, P. F., James, D. E. & Martin, J. L. (2007). *Acta Cryst. F* **63**, 524–528.
- Lee, H. O., Byun, Y. J., Cho, K.-O., Kim, S. Y., Lee, S.-B., Kim, H.-S., Kwon, O.-J. & Jeong, S.-W. (2011). *Korean J. Physiol. Pharmacol.* **15**, 149–156.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- McNew, J. A., Coe, J. G., Søgaard, M., Zemelman, B. V., Wimmer, C., Hong, W. & Söllner, T. H. (1998). *FEBS Lett.* **435**, 89–95.
- McNew, J. A., Søgaard, M., Lampen, N. M., Machida, S., Ye, R. R., Lacomis, L., Tempst, P., Rothman, J. E. & Söllner, T. H. (1997). *J. Biol. Chem.* **272**, 17776–17783.
- Misura, K. M., Bock, J. B., Gonzalez, L. C. Jr, Scheller, R. H. & Weis, W. I. (2002). *Proc. Natl Acad. Sci. USA*, **99**, 9184–9189.
- Munson, M., Chen, X., Cocina, A. E., Schultz, S. M. & Hughson, F. M. (2000). *Nature Struct. Biol.* **7**, 894–902.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Parlati, F., Varlamov, O., Paz, K., McNew, J. A., Hurtado, D., Söllner, T. H. & Rothman, J. E. (2002). *Proc. Natl Acad. Sci. USA*, **99**, 5424–5429.
- Pelham, H. R. B. (1999). *Exp. Cell Res.* **247**, 1–8.
- Sutton, R. B., Fasshauer, D., Jahn, R. & Brunger, A. T. (1998). *Nature (London)*, **395**, 347–353.
- Tsui, M. M. K. & Banfield, D. K. (2000). *J. Cell Sci.* **113**, 145–152.