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Crystallization and X-ray analysis of bovine glycolipid transfer protein

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Glycolipid-transfer protein (GLTP) is a 24 kDa basic cytosolic protein that facilitates the transfer of glycolipids between bilayer membranes *in vitro*, but its *in vivo* function is unknown. Human, bovine, porcine and murine GLTPs have recently been cloned and share high sequence identity to each other. The three-dimensional structure of GLTP has not yet been solved and no structures of any proteins related to GLTP are known. Therefore, the structure of GLTP might reveal a currently unknown fold. Here, the crystallization and preliminary X-ray analysis of bovine GLTP are reported for the first time. Protein prepared by recombinant techniques using an *Escherichia coli* expression system has been crystallized using the vapour-diffusion method. The crystals belong to space group $P2_1$, with unit-cell parameters a = 55.4, b = 34.9, c = 58.5 Å, $\alpha = \gamma = 90$, $\beta = 116^\circ$. The crystals diffract to 1.6 Å resolution and a 97.1% complete data set with an $R_{\rm merge}$ of 6.7% has been collected from a single crystal at 100 K using synchrotron radiation.

1. Introduction

Glycolipid-transfer protein (GLTP) facilitates the transfer of glycolipids between bilayer membranes *in vitro*; however, its biological function is not known. Bovine, porcine, human and murine GLTPs have recently been cloned and identified (Lin *et al.*, 2000). All these 24 kDa basic (pI 9.0) cytosolic proteins are extremely similar at the amino-acid sequence level; bovine and porcine GLTPs share an identical 209-amino-acid sequence, while human and murine GLTPs are 98 and 91% identical to bovine GLTP, respectively. Several characteristics of bovine and porcine brain GLTPs suggest that these proteins clearly differ from other known lipid-transfer proteins (Bankaitis *et al.*, 1996).

In the filamentous fungi *Podospora anserina*, a 24 kDa gene product called HET-C2 shows glycosphingolipid-transfer activity and GLTP-like characteristics (Mattjus *et al.*, 2003). HET-C2 is involved in regulating cell-compatibility interactions during heterokaryon fusion (Saupe *et al.*, 1994). Fusion of compatible cells leads to ascospore production, whereas fusion of incompatible cells triggers a process analogous to apoptosis. Recently, a GLTP-like protein has been identified from *Arabidopsis thaliana* (Brodersen *et al.*, 2002). This 22.7 kDa protein, called ACD11 (accelerated cell death 11), does not transfer glycosphingolipids *in vitro*; instead, it facilitates the intermembrane transfer of single-chain sphingosine. The lethal recessive ACD11 knockout in *A. thaliana* causes defence activation and activation of programmed cell death analogous to animal cell apoptosis (Brodersen *et al.*, 2002). HET-C2 and ACD11 share ∼27 and ∼16% sequence identity with GLTP, respectively. Since ACD11 and HET-C2 are homologous to GLTP and have similar *in vitro* transfer activities, their role in the cell death response of multicellular eukaryotes might indicate a similar physiological role for the emerging family of sphingolipid-transfer proteins.

The crystal structures of two proteins with glycolipid-transfer activity have been solved: GM2 activator protein (PDB code 1g13; Wright *et al.*, 2000) and rabbit sterol carrier protein 2 (SCP-2; PDB code 1c44; Choinowski *et al.*, 1999, 2000). However, these proteins do not share any sequence similarity with GLTP. The 2.0 Å resolution crystal structure of GM2 activator protein revealed a new fold type with an eight-stranded cup-shaped antiparallel β-pleated sheet (Wright *et al.*, 2000), whereas the 1.8 Å crystal structure of rabbit SCP-2 showed a unique α/β-fold in which the core of the protein forms a five-stranded antiparallel β-sheet flanked by five helices (Choinowski *et al.*, 1999, 2000). GM2 activator protein binds and transfers ganglioside GM2 and asialo-GM2 (Conzelmann *et al.*, 1982), while several SCP-2s identified in both animal and plant sources have been shown to catalyze the *in vitro* transfer of a wide range of lipids,

including glycolipids (Wirtz, 1997), various neutral glycosphingolipids and ganglioside GM1 (Bloj & Zilversmit, 1981).

Here, we report the crystallization and preliminary X-ray analysis of bovine GLTP. No proteins with sequence homology to GLTP have previously been crystallized. The data collected and presented here will serve as a basis for determining the crystal structure of GLTP. The structure would provide valuable information about its unknown ligand-binding site and reveal the structural determinants of glycolipid specificity.

2. Materials and methods

2.1. Expression and purification

The bovine GLTP gene (Lin *et al.*, 2000) has previously been cloned into a pQE-9 vector utilizing the N-terminal 6×His fusion-protein construct (Qiagen). The vector was transformed into *Escherichia coli* BL21 cells and grown in LB medium at 310 K until the cell density A_{600} reached 0.6. Expression of the GLTP-fusion protein was induced by addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 m*M*. After 2 h incubation, the cells were harvested and lysed by incubation with lysozyme in lysis buffer (50 m*M* NaH_2PO_4, 300 m*M* NaCl, 10 m*M* imidazole and 10% glycerol pH 8.0) followed by sonication. The clarified lysate was purified twice by affinity chromatography on a column packed with Ni–NTA agarose beads (Qiagen). The protein was eluted with elution buffer (50 m*M* NaH_2PO_4, 300 m*M* NaCl, 250 m*M* imidazole and 10% glycerol pH 8.0). The purity was confirmed by analysis on an SDS–PAGE gel (16%) and Coomassie staining. The GLTP was concentrated to 17.5–24 mg ml^{-1} with a protein concentrator (Millipore). Both the Lowry and Bradford methods were used to determine the protein concentration (Lowry *et al.*, 1951; Bradford, 1976).

2.2. Crystallization and preliminary X-ray analysis

Initial crystallization conditions for GLTP were screened at 280 K using the Crystal Screen I (Hampton Research Inc.) random sparse-matrix crystallization screen and the vapour-diffusion method. Small crystals were obtained from a condition consisting of 30%(*w*/*v*) PEG 8000, 100 m*M* MES pH 6.6 and 200 m*M* sodium acetate. The hanging drops contained 2 µl protein sample (∼20 mg ml^{-1}) in elution buffer and 2 µl well solution. The precipitant concentration and the pH of the MES buffer were optimized and the best crystals were obtained using a reservoir solution consisting of 25–30% PEG 8000, 0.1 *M* sodium acetate and 0.1 *M* MES pH 5.6–6.0 as the precipitant. The actual pH of the drop was 8.0. The crystals formed in a few days and grew to average dimensions of 0.75 × 0.1 × 0.1 mm (Fig. 1). X-ray analysis and data collection were carried out using synchrotron radiation at beamline X13, EMBL/DESY Hamburg, Germany equipped with a bent mirror, a triangular monochromator and a MAR Research CCD detector. For data collection, the crystals were cryoprotected with 20%(*v*/*v*) glycerol and flash-frozen in a 100 K nitrogen stream. A complete data set was collected from a single crystal using dose mode (about 100 s exposure time), an oscillation range of 0.5° and a crystal-to-detector distance of 145 mm using MAR Research software. Diffraction data were processed with the program *XDS* (Kabsch, 1993) using the default σ cutoff of −3.0 for the reflections. The solvent content and Matthews coefficient were calculated assuming a molecular weight of 24 kDa using the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

Table 1
Crystal and diffraction data statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 55.4, b = 34.9, c = 58.5, $\alpha = \gamma = 90$, β = 116
Matthews coefficient (Å^3 Da^{-1})	2.1
Solvent content (%)	41
Unit-cell volume (Å^3)	100949
Molecules per asymmetric unit	1
Resolution range (Å)	20–1.61 (1.70–1.61)
Wavelength used (Å)	0.804
Unique reflections	25509 (3717)
Observed reflections	105133 (14079)
Completeness (%)	97.1 (95.1)
R_{merge} (%)	6.7 (41.4)
Average $I/\sigma(I)$	12.16 (3.27)
Redundancy	4.1 (3.8)

3. Results and discussion

Here, we report for the first time the crystallization and preliminary X-ray analysis of bovine GLTP. The full-length protein was expressed as a His-tagged fusion protein in BL-21 *E. coli* cells and affinity purified with metal-affinity chromatography. The typical yield was 4–5 mg per litre of bacterial culture and the purity was judged to be about 90–95% homogeneous based on either Coomassie or silver staining. The protein retained glycolipid-transfer activity as measured by a transfer assay (Mattjus *et al.*, 1999, 2000). The highest quality crystals were obtained using 25–30% PEG 8000, 0.1 *M* sodium acetate and 0.1 *M* MES pH 5.6–6.0 as the precipitant. The crystals grew

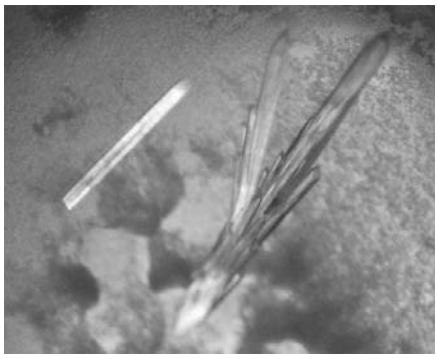

Figure 1
GLTP crystals.

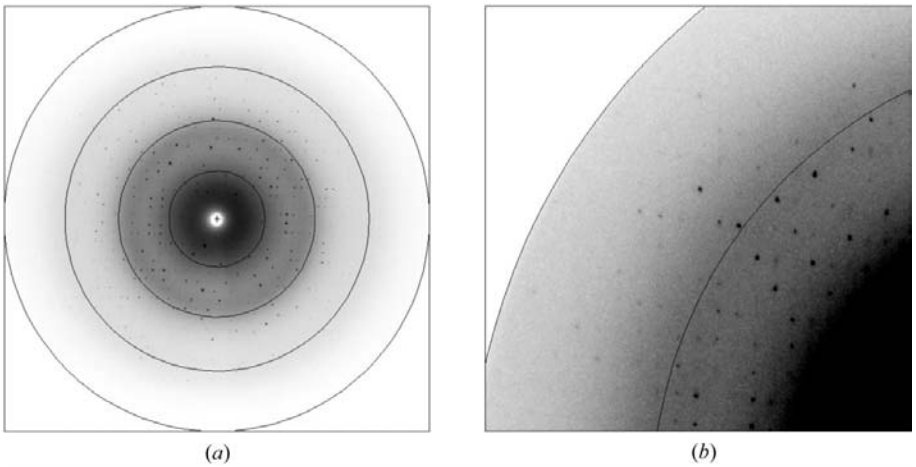

Figure 2
(*a*) A typical GLTP diffraction pattern and (*b*) a magnified view. Resolution rings correspond to 1.6, 2.1, 3.2 and 6.4 Å.

to typical dimensions of $0.75 \times 0.1 \times 0.1$ mm (Fig. 1) and diffracted to 1.6 Å resolution. No radiation damage was detected during data collection. The crystals belonged to space group $P2_1$, with unit-cell parameters $a = 55.4$, $b = 34.9$, $c = 58.5$ Å, $\alpha = \gamma = 90$, $\beta = 116^\circ$ (Fig. 2), and had a mosaicity of 0.3. If we assume the presence of one molecule per asymmetric unit, then the Matthews coefficient is 2.1 $\text{Å}^3\,\text{Da}^{-1}$ and the solvent content is 41%. Crystal parameters and diffraction data statistics are summarized in Table 1.

The crystal structure of GLTP may reveal a novel fold since no structures from any protein related to GLTP are known to date. The structure would reveal the lipid-binding site in detail and would provide an insight into the binding properties as well as the lipid recognition and specificity of GLTP. Thus, the structure of GLTP would be of great importance in understanding its biological role. The data presented here provide a basis for determining the crystal structure of the soluble bovine GLTP. The structure will be solved by the multiple anomalous dispersion method using selenomethionine-derivative crystals. The structure determination is currently in progress.

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References

Bankaitis, V. A., Fry, M. R., Cartee, R. T. & Kagiwada, S. (1996). *Phospholipid Transfer Proteins: Emerging Roles in Vesicle Trafficking, Signal Transduction and Metabolic Regulation*, pp. 51–72. Austin, USA: Landes Bioscience.

Bloj, B. & Zilversmit, D. B. (1981). *J. Biol. Chem.* **256**, 5988–5991.

Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.

Brodersen, P., Petersen, M., Pike, H. M., Olszak, B., Skov, S., Odum, N., Jorgensen, L. B., Brown, R. E. & Mundy, J. (2002). *Genes Dev.* **16**, 490–502.

Choinowski, T., Dyer, J. H., Maderegger, B., Winterhalter, K. H., Hauser, H. & Piontek, K. (1999). *Acta Cryst.* D**55**, 1478–1480.

Choinowski, T., Hauser, H. & Piontek, K. (2000). *Biochemistry*, **39**, 1897–1902.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Conzelmann, E., Burg, J., Stephan, G. & Sandhoff, K. (1982). *Eur. J. Biochem.* **123**, 455–464.

Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.

Lin, X., Mattjus, P., Pike, H. M., Windebank, A. J. & Brown, R. E. (2000). *J. Biol. Chem.* **275**, 5104–5110.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.

Mattjus, P., Molotkovsky, J. G., Smaby, J. M. & Brown, R. E. (1999). *Anal. Biochem.* **268**, 297–304.

Mattjus, P., Pike, H. M., Molotkovsky, J. G. & Brown, R. E. (2000). *Biochemistry*, **39**, 1067–1075.

Mattjus, P., Turcq, B., Pike, H. M., Molotkovsky, J. G. & Brown, R. E. (2003). *Biochemistry*, **42**, 535–542.

Saupe, S., Descamps, C., Turcq, B. & Begueret, J. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 5927–5931.

Wirtz, K. W. (1997). *Biochem. J.* **324**, 353–360.

Wright, C. S., Li, S. C. & Rastinejad, F. (2000). *J. Mol. Biol.* **304**, 411–422.