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Crystallization and preliminary X-ray crystallographic analysis of human RGS10 complexed with Gαi3

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G-protein-coupled receptors, which are major targets for drug discovery, play a major role in diverse physiological processes by relating changes in the extracellular environment to intracellular functions *via* activation of heterotrimeric G-proteins. However, G-protein activity is also modulated by a family of proteins called regulators of G-protein signalling (RGS), which are classified into six subfamilies. RGS10 belongs to the subgroup D/R12 and is known to act specifically on activated forms of three Gα proteins (Gαi3, Gαz and Gαo but not Gαs). It is abundantly expressed in brain and immune tissues and has been implicated in the pathophysiology of schizophrenia. The RGS domain of RGS10 was cloned, purified, complexed with human Gαi3 and crystallized. The crystals containing both RGS and Gαi3 belong to space group $P4_32_12$ (or $P4_12_12$), with unit-cell parameters $a = 99.88$, $b = 99.88$, $c = 144.59$ Å, $\alpha = \beta = \gamma = 90^\circ$. A full set of diffraction data were collected to 2.5 Å resolution at 100 K using synchrotron radiation at Pohang beamline 4A.

1. Introduction

Signal transduction is a fundamental biological process relating changes in the extracellular environment to changes in intracellular function. The vast majority of receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), which forms one of the largest protein families found in nature. A diverse array of stimuli including neurotransmitters, hormones, lipids, calcium ions and many others can act through GPCRs, giving rise to remarkably diverse physiological functions. As such, GPCRs are the targets of many therapeutic drugs. In GPCR signalling, stimulation of the seven transmembrane helical receptors leads to the activation of heterotrimeric G-proteins, which dissociate into α and $\beta\gamma$ subunits (Gilman, 1987). The Gα subunit, which has been classified into four families (Gαs, Gαi/o, Gαq and Gα12), has guanine nucleotide-binding and GTP-hydrolysis activities (Simon *et al.*, 1991; Hepler & Gilman, 1992).

Precise control of these processes is of great importance in both the short and long term. The regulator of G-protein signalling (RGS) proteins serve as GTPase-activating proteins that accelerate GTP hydrolysis and thereby return the Gα subunit to its inactivated GDP-bound form. RGS and RGS-like proteins are a family at least of 25 proteins and have been classified into six subfamilies based on the alignment of RGS domains and amino-acid sequences (Zheng *et al.*, 1999; Ross & Wilkie, 2000). They all contain a conserved RGS box, which is about 120 amino acids long and is responsible for binding the Gα protein, often accompanied by other signalling regulatory elements. RGS proteins accelerate the deactivation of G-proteins to reduce GPCR signalling; however, some have also been shown to have an effector function and transmit signals. Based on this, it has been suggested that combining GPCR agonists with RGS inhibitors might potentiate responses and could markedly increase the agonist's regional specificity (De Vries *et al.*, 2000; Zhong & Neubig, 2001; Brady & Limbird, 2002; Hollinger & Hepler, 2002; Neubig & Siderovski, 2002; Wieland & Mittmann, 2003 and references therein).

In order to elucidate the mechanistic basis of the function of RGS in G-protein signalling pathways and to further develop inhibitors, it is essential to obtain detailed structural information. The crystal structures of RGS4–Gαi1, RGS9–Gαt/i1–PDEγ and the rgRGS

domain of p115RhoGEF–Gα13/i1 chimera complexes have been reported (Tesmer *et al.*, 1997; Slep *et al.*, 2001; Chen *et al.*, 2005). Here, we report structural experiments on the complex of RGS10 with Gαi3. RGS10 consists of 173 amino acids, belongs to the subgroup D/R12 and is known to act specifically on activated forms of three G-protein subunits, Gαi3, Gαz and Gαo, but not on Gαs. RGS10 is abundantly expressed in brain and in immune tissues and has been implicated in diverse processes including modulation of presynaptic and postsynaptic G-protein signalling as well as a possible role in regulation of gene expression (Hunt *et al.*, 1996; Popov *et al.*, 1997; Chatterjee & Fisher, 2000; Waugh *et al.*, 2005).

2. Experimental methods

2.1. Construction and expression

The human *Gnai3* gene, which encodes a 353-residue protein (M_r = 40530 Da), was isolated from human foetal liver cDNA and expressed using the pET28a vector. The construction added 20 residues including a hexahistidine tag to the N-terminus of the recombinant Gαi3. The human *rgs10* full-length gene was isolated from the human cDNA library and the RGS domain (residues 21–173) was subcloned. The gene encodes a 153-residue (M_r = 18238 Da) protein. The designed forward and reverse oligonucleotide primers were 5′-GGG AAT TCC ATA TGC ACC AGA GCC TCA AGA GCA CA-3′ and 5′-CGC CGC CCG CTC GAG TGT GTT ATA AAT TCT GGA AGC-3′, encoding *Nde*I and *Xho*I restriction-endonuclease sites. For amplication, 1 µg DNA template was used with 0.2 m*M* dNTP (TaKaRa, Japan), 1 unit of Ex Taq polymerase (TaKaRa, Japan) and 0.5 µ*M* primer. 30 reaction cycles of 30 s at 367 K/30 s at 328 K/60 s at 345 K were carried out followed by an additional 7 min incubation at 345 K in T-gradient thermo-blocks (Biometra, Germany). The PCR product was purified using the Bio101 Gene Clean kit (Bio101, USA). After the PCR product had been digested using *Nde*I and *Xho*I enzymes, it was inserted into the *Nde*I/*Xho*I-digested expression vector pET22b. This construction added a hexahistidine tag to the C-terminus of the recombinant RGS10.

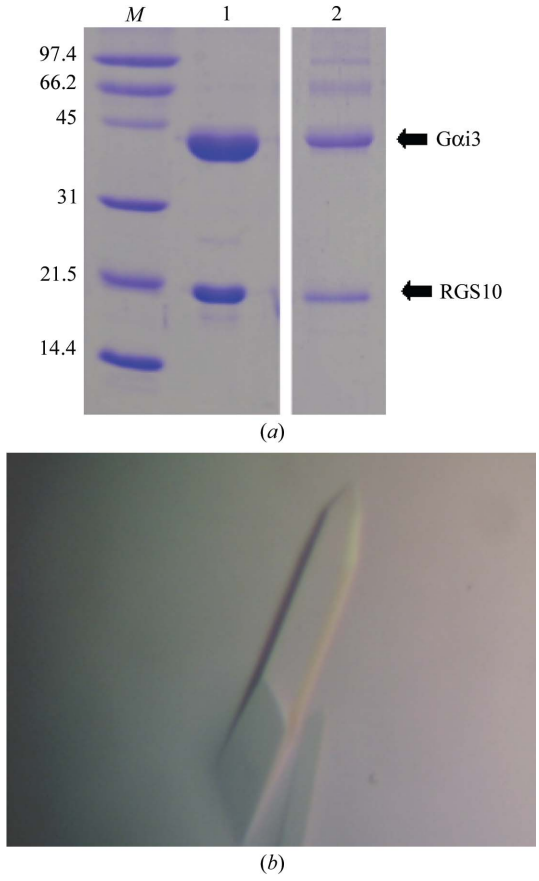

Figure 1
(*a*) SDS–PAGE analysis of purified RGS10 and Gαi3 after the gel-filtration column step (lane 1) and of dissolved crystals (lane 2). Lane *M* contains molecular-weight markers (kDa). (*b*) A crystal of the complex of the RGS domain of human RGS10 and human Gαi3. Its approximate dimensions are 0.3 × 0.09 × 0.1 mm.

RGS10 and Gαi3 were overexpressed separately in *Escherichia coli* BL21(DE3) cells. The cells were grown in Luria–Bertani (LB) medium in the presence of antibiotics (50 µg ml^{-1} ampicillin in the case of RGS10 and 10 µg ml^{-1} kanamycin in the case of Gαi3) to an OD_{600} of 0.5 at 310 K and expression of the recombinant protein was induced by adding 0.5 m*M* isopropyl *β*-D-thiogalactopyranoside (IPTG) at 291 K. Cell growth continued at 291 K for 16 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 30 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.6, 300 m*M* NaCl and 5% glycerol) and homogenized by sonification. The crude lysate was centrifuged for 30 min at 277 K and the supernatant was passed through a 0.45 µm filter to remove cell debris.

2.2. Purification and complex formation

For both RGS10 and Gαi3, the first step in purification utilized the histidine tag by using a nickel-chelated Hi-trap chelating column (Amersham Biosciences). In both cases, reasonable purity was obtained after this step and purified RGS10 and Gαi3 were mixed in a 1:1 molar ratio and incubated in 20 m*M* Tris–HCl pH 7.6, 300 m*M* NaCl, 5% glycerol, 2 m*M* *β*-mercaptoethanol, 10 m*M* DTT, 16 m*M* $MgCl_2$, 32 m*M* NaF, 64 µ*M* $AlCl_3$, 30 µ*M* GDP and 2 m*M* EDTA for 15 min at 295 K. After incubation, a gel-filtration step using a Superdex-200 prep-grade column (Amersham Biosciences) was carried out. The column was pre-equilibrated with a buffer containing 20 m*M* HEPES pH 8.0, 100 m*M* NaCl, 5 m*M* $MgCl_2$, 2 m*M* DTT, 1 m*M* EDTA, 30 µ*M* $AlCl_3$, 20 µ*M* GDP and 20 m*M* NaF. The purified protein solution was concentrated to 37 mg ml^{-1} using an Amicon Ultra-15 centrifugal ultrafiltration unit (Millipore). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient 5120 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/). The purity of protein was monitored by SDS–PAGE using Coomassie blue staining (Fig. 1*a*).

2.3. Crystallization

Initial screening for crystallization conditions was carried out by the sitting-drop vapour-diffusion method using 96-well Intelli plates (Hampton Research) and a Hydra II Plus One system (Matrix Technology) at 295 K. The protein concentration was 37 mg ml^{-1} in 20 m*M* HEPES pH 8.0, 100 m*M* NaCl, 5 m*M* $MgCl_2$, 2 m*M* DTT, 1 m*M* EDTA, 30 µ*M* $AlCl_3$, 20 µ*M* GDP and 20 m*M* NaF. A sitting drop was prepared by mixing 200 nl each of the protein solution and the reservoir solution and was equilibrated against 70 µl reservoir solution. The initial search for crystallization conditions was performed using commercially available kits from Hampton Research, Jena Bioscience and Emerald Biostructures. Of 1200 conditions screened, several microcrystals were obtained after 1–5 d: thin needle clusters were obtained from Hampton Index Screen condition Nos. 20 (25% PEG 3350, 0.1 *M* HEPES pH 7.5, 0.2 *M* ammonium sulfate) and 19 (25% PEG 3350, 0.1 *M* bis-tris pH 6.5,

Table 1
Summary of crystallographic data.

Values in parentheses are for the outermost resolution shell (2.59–2.50 Å).

X-ray wavelength (Å)	0.97123 (Pohang beamline 4A)
Resolution range (Å)	30–2.5
Space group	$P4_32_12$ or $P4_12_12$
Unit-cell parameters (Å)	a = 99.88, b = 99.88, c = 144.59
Total/unique reflections	287950/25992
Completeness (%)	99.7 (99.1)
Mean $I/\sigma(I)$ (%)	26.2 (4.4)
$R_{\rm merge}$† (%)	0.080 (0.299)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h,i)$ for all i measurements.

0.2 *M* ammonium sulfate) and JBS Screen 6 No. 15 (5% PEG 400, 0.1 *M* Na MES pH 6.5, 2 *M* ammonium sulfate).

These conditions were optimized using hanging-drop vapour-diffusion experiments. Each hanging drop was prepared by mixing 1 µl each of the protein solution and the reservoir solution and was equilibrated over 0.5 ml reservoir solution. Diffraction-quality crystals were obtained with a reservoir solution consisting of 22% PEG 3350, 0.1 *M* bis-tris pH 6.5, 0.2 *M* ammonium sulfate. In order to confirm that the crystals were of the complex, we harvested several crystals, washed them with crystallization buffer and dissolved them in SDS buffer. SDS–PAGE analysis (Fig. 1*a*) of the dissolved crystals confirmed the presence of both RGS10 and Gαi3 proteins in the crystal.

2.4. Data collection

A RGS10–Gαi3 complex crystal measuring 0.3 × 0.09 × 0.1 mm grown in 3 d using 22% PEG 3350, 0.1 *M* bis-tris pH 6.5, 0.2 *M* ammonium sulfate (Fig. 1*b*) was used for data collection. The crystal mounted in a cryoloop was dipped in cryoprotectant solution containing 2% PEG 6000, 0.1 *M* MES pH 5.5 and 30%(*v*/*v*) ethylene glycol for 2 s and immediately transferred to a nitrogen stream at 100 K. Diffraction data were collected from the crystal at 100 K using an ADSC Quantum CCD Q210 detector (ADSC, USA) at beamline 4A of Pohang Light Source. The wavelength used was 0.97123 Å. The crystal was rotated through a total of 180°, with 1.0° oscillation per frame and 5 s exposure time per frame. The raw data were processed and scaled using the program *HKL*2000 (Otwinowski & Minor, 1997; HKL Research Inc.).

3. Results

The crystal belongs to space group $P4_32_12$ (or $P4_12_12$), with unit-cell parameters a = 99.88, b = 99.88, c = 144.59 Å, $\alpha = \beta = \gamma = 90°$. The native data set is 99.7% complete to 2.5 Å resolution. The presence of one molecule of RGS and Gαi3 in the asymmetric unit gives a crystal volume per protein weight ($V_{\rm M}$) of 3.07 Å^3 Da^{-1}, with a corresponding solvent content of 60.0% (Matthews, 1968). Data-collection statistics are summarized in Table 1. Structure determination and refinement is currently in progress.

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References

Brady, A. E. & Limbird, L. E. (2002). *Cell. Signal.* **14**, 297–309.
Chatterjee, T. K. & Fisher, R. A. (2000). *J. Biol. Chem.* **275**, 24013–24021.
Chen, Z., Singer, W. D., Sternweis, P. C. & Sprang, S. R. (2005). *Nature Struct. Mol. Biol.* **12**, 191–197.
De Vries, L., Zheng, B., Fischer, T., Elenko, E. & Farquhar, M. G. (2000). *Annu. Rev. Pharmacol. Toxicol.* **40**, 235–271.
Gilman, A. G. (1987). *Annu. Rev. Biochem.* **56**, 615–649.
Hepler, J. R. & Gilman, A. G. (1992). *Trends Biochem. Sci.* **17**, 383–387.
Hollinger, S. & Hepler, J. R. (2002). *Pharnacol. Rev.* **54**, 527–559.
Hunt, T. W., Fields, T. A., Casey, P. J. & Peralta, E. G. (1996). *Nature (London)*, **383**, 175–177.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Neubig, R. R. & Siderovski, D. P. (2002). *Nature Rev. Drug Discov.* **1**, 187–197.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–332.
Popov, S., Yu, K., Kozasa, T. & Wilkie, T. M. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 7216–7220.
Ross, E. M. & Wilkie, T. M. (2000). *Annu. Rev. Biochem.* **69**, 795–827.
Simon, M. I., Strathmann, M. P. & Gaulam, N. (1991). *Science*, **252**, 4037–4041.
Slep, K. C., Kercher, M. A., He, W., Cowan, C. W., Wensel, T. G. & Sigler, P. B. (2001). *Nature (London)*, **409**, 1071–1077.
Tesmer, J. J., Berman, D. M., Gilman, A. G. & Sprang, S. R. (1997). *Cell*, **89**, 251–261.
Waugh, J. L., Lou, A. C., Eisch, A. J., Monteggia, L. M., Muly, E. C. & Gold, S. J. (2005). *J. Comp. Neurol.* **481**, 299–313.
Wieland, T. & Mittmann, C. (2003). *Pharmacol. Ther.* **97**, 95–115.
Zheng, B., De Vries, L. & Gist Farquhar, M. (1999). *Trends Biochem. Sci.* **24**, 411–414.
Zhong, H. & Neubig, R. R. (2001). *J. Pharmacol. Exp. Ther.* **279**, 837–845.