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ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 831–838
BBRC
www.elsevier.com/locate/ybbrc

High resolution structures of the bone morphogenetic protein type II receptor in two crystal forms: Implications for ligand binding

Peter D. Mace, John F. Cutfield, Sue M. Cutfield *

Department of Biochemistry, Otago School of Medical Sciences, University of Otago, P.O. Box 56, Dunedin 9001, New Zealand

Received 17 October 2006

Abstract

BMPRII is a type II TGF-β serine threonine kinase receptor which is integral to the bone morphogenetic protein (BMP) signalling pathway. It is known to bind BMP and growth differentiation factor (GDF) ligands, and has overlapping ligand specificity with the activin type II receptor, ActRII. In contrast to activin and TGF-β type ligands, BMPs bind to type II receptors with lower affinity than type I receptors. Crystals of the BMPRII ectodomain were grown in two different forms, both of which diffracted to high resolution. The tetragonal form exhibited some disorder, whereas the entire polypeptide was seen in the orthorhombic form. The two structures retain the basic three-finger toxin fold of other TGF-β receptor ectodomains, and share the main hydrophobic patch used by ActRII to bind various ligands. However, they present different conformations of the A-loop at the periphery of the proposed ligand-binding interface, in conjunction with rearrangement of a disulfide bridge within the loop. This particular disulfide (Cys94–Cys117) is only present in BMPRII and activin receptors, suggesting that it is important for their likely shared mode of binding. Evidence is presented that the two crystal forms represent ligand-bound and free conformations of BMPRII. Comparison with the solved structure of ActRII bound to BMP2 suggests that His87, unique amongst TGF-β receptors, may play a key role in ligand recognition.

Keywords: BMP; TGF-β family; Type II serine threonine kinase receptor; Three-finger toxin; Ligand binding

The transforming growth factor β (TGF-β) superfamily of cytokines is a large group of structurally related proteins that include activins, inhibins, anti-Müllerian hormone (AMH), bone morphogenetic proteins (BMPs) as well as TGF-βs themselves. Active TGF-β dimers elicit their effects by interacting with two types of receptors. Type I and type II TGF-β receptors possess a cysteine-rich extracellular domain, a single transmembrane segment, and a cytoplasmic serine threonine kinase domain. Signal transduction occurs when the kinase domain of the type II receptor phosphorylates the type I receptor, which in turn activates a SMAD signalling cascade [1]. Type I receptors possess an additional intracellular glycine-serine-rich domain that is essential for signal transduction as a substrate for phosphorylation [2]. Seven type I and five type II mammalian receptors have been identified, whereas there are more than 40 TGF-β ligands [3–6]. This imbalance suggests an intriguing scenario, where a single receptor may interact with multiple structurally similar ligands in distinct ways. Increasing signalling complexity further, ligands are able to dimerise *in vivo* as homo- or hetero-dimeric species [7], and receptors may associate as polygamous pairs [8]. Whatever the components, specificity must be generated via sequence-specific regions and conformational flexibility, given a common structural scaffold for both receptors and ligands.

Within the TGF-β superfamily, BMPs comprise a group of over 20 proteins, and were first identified through their ability to induce ectopic bone and cartilage formation [9]. Growth and differentiation factors (GDFs) are included as a subset of BMPs, and both have been shown to play important roles with respect to embryonal pattern formation, and growth, development, and survival of a wide

* Corresponding author.
E-mail address: sue.cutfield@otago.ac.nz (S.M. Cutfield).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.10.109

range of cell types [3]. Mice lacking individual type I BMP receptors also have severe developmental abnormalities [10,11], while the type II BMP receptor (BMPRII) is essential for epiblast differentiation and mesoderm induction such that ablation of the gene arrests development at an early stage [12]. Furthermore, human mutations in BMPRII have been detected in patients suffering familial pulmonary hypertension [13].

While both type II activin receptors (ActRII and ActRIIb) have been shown to bind BMP ligands as well as activins, BMPRII binds exclusively to BMP ligands, including BMP2, BMP4, BMP6, BMP7, BMP15, GDF5, and GDF9 [6]. It is thought that BMP ligands have much lower affinity for BMPRII than for their type I receptors [14,15]. It has been proposed that the two receptor types may act together to form a high affinity complex for BMP ligands [16,17], or alternatively type I receptors may first bind to ligand followed by recruitment of BMPRII [16,17]. This contrasts with TGF-βs and activins, where high affinity type II receptors first bind ligand and subsequently recruit type I receptors.

Receptor ectodomains for which structures have been determined include BMPRIa [18,19], TGF-βRII [20,21], ActRII [22,23] and ActRIIb [24,25]. Structures of binary complexes between ligands and receptor ectodomains have been solved for TGF-βRII:TGF-β3 [21], ActRII:BMP7 [23], ActRII:activin A [24,25], and BMPRIa:BMP2 [18,19], and recently a ternary structure has been reported for BMP2 in complex with both a type I (BMPRIa) and a type II receptor (ActRII) [22]. Here, we report the structure of ovine BMPRII solved to high resolution in two crystal forms, and compare it with other TGF-β family receptors, gleaning insight into possible ligand-binding interactions. The amino acid sequence of the ovine ectodomain is identical to human and to other mammalian species, and considering the diverse processes with which BMPRII is involved, these results have wide relevance.

Materials and methods

Protein expression and purification. Ovine BMPRII (residues 32–131) was expressed as inclusion bodies in *Escherichia coli*, refolded, and purified as previously described [26]. In short, refolded BMPRII was purified by anion exchange using Q-Sepharose, and monomeric BMPRII was separated using a HiLoad Superdex-75 size-exclusion column. Final purification was achieved by applying a NaCl gradient to a MonoP column.

Crystallisation and data-collection. Diffraction quality crystals of BMPRII were grown in two different space groups by hanging-drop vapour diffusion at 18 °C, using BMPRII(32–131) at a concentration of 10 mg/mL in 20 mM Hepes (pH 7.0) and 50 mM NaCl.

Tetragonal crystals were prepared by mixing 500 nL of protein solution with 250 nL of 25% PEG2000 MME, 0.8 M sodium formate, and 0.1 M sodium citrate (pH 5.6). Crystals appeared after 1–4 weeks as rectangular blocks. For data-collection, crystals were flash-frozen directly from mother liquor in liquid nitrogen at 110 K. A mercury derivative was prepared by soaking a single crystal in $HgCl_2$ saturated mother liquor for 30 min prior to freezing. These crystals proved to be in space group $P4_32_12$ with cell dimensions (Å) of $a = b = 34.88$, $c = 120.46$, $\alpha = \beta = \gamma = 90°$, with 1 molecule of BMPRII in the asymmetric unit. Data from the mercury derivative were collected using a copper rotating anode X-ray source, while a high-resolution native dataset was collected at SSRL beamline BL9-2 at a wavelength of 0.97946 Å. All images were integrated using MOSFLM [27] and processed with SCALA [28] and programs from the CCP4 suite [29].

Orthorhombic crystals were prepared by mixing 500 nL of protein solution with 250 nL of 15% PEG1500, 0.2 M magnesium acetate, and 0.1 M sodium citrate (pH 6.0). Crystals appeared after 1–2 days and possessed a rod-shaped morphology. For data-collection, crystals were soaked in mother-liquor containing an additional 10% (v/v) glycerol and flash-frozen in liquid–nitrogen at 110 K. These crystals proved to be in space group $P2_12_12_1$ with cell dimensions (Å) of $a = 35.63$, $b = 45.01$, $c = 48.06$, $\alpha = \beta = \gamma = 90°$ with 1 molecule of BMPRII in the asymmetric unit. A highly redundant dataset was initially collected using a copper rotating anode X-ray source, while a high resolution dataset was later collected from the same crystal at SSRL beamline BL9-1 using a wavelength of 0.97946 Å. Tetragonal crystals also grew under the same conditions that gave rise to orthorhombic crystals. Diffraction data statistics are presented in Table 1.

Other methods. Biological activity of recombinant protein was assessed by the ability of receptor ectodomain to inhibit GDF9 and BMP15 stimulated mitosis of rat granulosa cells using the method of McNatty et al. [30]. Structures were validated using SFCHECK [31] and PROCHECK [32], and overlayed using SSM-Superpose [33] via the COOT

Table 1
Summary of datasets used for structure solution

Dataset	Orthorhombic native	Orthorhombic native (HiRes)	Tetragonal native	Tetragonal mercury
Space group	$P2_12_12_1$	$P2_12_12_1$	$P4_32_12$	$P4_32_12$
Unit cell parameters				
a (Å)	35.63	35.58	35.05	34.88
b (Å)	45.01	45.07	35.05	34.88
c (Å)	48.06	48.16	121.04	120.46
Wavelength (Å)	1.54	0.97946	0.97946	1.54
Temperature (K)	113	110	110	113
Measured reflections	106,974	523,47	116,390	62,923
Unique reflections	5173	13746	23947	4143
Resolution range (Å)	18.22–2.03	32.92–1.45	40.32–1.20	30.18–2.20
Highest-resolution bin (Å)	2.14–2.03	1.53–1.45	1.26–1.20	2.32–2.20
Completeness (%)	96.5 (84.3)	96.7 (87.1)	97.3 (100)	98.1 (88.2)
Multiplicity	20.8 (19.8)	3.8 (3.7)	4.9 (4.5)	15.2 (13.6)
R_{merge}	0.049 (0.120)	0.053 (0.378)	0.055 (0.376)	0.091 (0.585)
$I/\sigma I$	52.2 (25.0)	13.7 (3.5)	16.2 (2.9)	29.5 (5.8)