Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of the unliganded human growth hormone receptor

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Received 16 August 2004
Accepted 28 October 2004

The crystal structure of the extracellular domain of growth hormone receptor complexed to its ligand, growth hormone, has been known since 1992. However, no information exists for the unliganded form of the receptor. The human growth hormone receptor's extracellular ligand-binding domain, encompassing amino-acid residues 1–238, has been expressed in *Escherichia coli*, purified by anion ion-exchange chromatography and crystallized in its unliganded state by the hanging-drop vapour-diffusion method in 100 m*M* HEPES pH 7.0 containing 27.5%(*w*/*v*) PEG 5000 monomethyl ether and 200 m*M* ammonium sulfate as the co-precipitants. The crystals belong to the othorhombic space group $C222_1$, have unit-cell parameters a = 99.7, b = 112.2, c = 93.2 Å and diffract to 2.5 Å resolution using synchrotron radiation. The crystal structure will shed light on the nature of any conformation changes that occur upon ligand binding and will provide information to develop potential low-molecular-weight agonists/antagonists to treat clinical diseases in which the growth hormone receptor is implicated.

1. Introduction

Growth hormone causes a diverse range of biological activities including bone growth, lactation, regulation of lipid and carbohydrate metabolism and actions on the reproductive and immune systems (Silva *et al.*, 1998). In order to accomplish these actions, growth hormone must bind to its specific cell-surface receptor to activate the requisite intracellular signal transduction pathways. The growth hormone receptor is a type I transmembrane receptor that belongs to the cytokine/haemopoietin receptor superfamily (Bazan, 1990). This family of receptors is characterized by the presence of fibronectin type III modules, conserved cysteine residues, a cytokine-receptor homology region and the WS*X*WS motif within the extracellular ligand-binding domain (Wells & de Vos, 1996). The intracellular domains of these receptors lack intrinsic kinase activity; instead, they contain a proline-rich region that serves as a binding site for a family of cytoplasmic tyrosine kinases called Janus kinases (JAKs). Human growth hormone exists as a monomer and has two binding sites for the extracellular domain of the growth hormone receptor. It is believed that ligand binding induces receptor dimerization and activation of JAKs by transphosphorylation. (Fuh *et al.*, 1992). Activated JAKs in turn phosphorylate tyrosine residues on the receptor's cytoplasmic domain and on downstream signalling molecules, resulting in altered cellular proliferation, differentiation, survival and metabolism depending on the cell type involved.

Recent evidence suggests that preformed dimers of the growth hormone receptor exist prior to ligand binding (Ross *et al.*, 2001; Gent *et al.*, 2002). We have expressed, purified and obtained crystals of the extracellular ligand-binding domain of the growth hormone receptor in the absence of growth hormone. This domain encompasses amino-acid residues 1−238 and the expressed protein has an apparent molecular weight of ∼30 kDa when analysed by SDS–PAGE. We anticipate that the crystal structure will shed light on how hormone binding to its receptor might lead to conformational change and receptor activation.

2. Experimental procedures and results

2.1. Cloning, expression and purification

The recombinant human growth hormone receptor extracellular ligand-binding domain (hGHBP) was expressed in *Escherichia coli* strain BL21-Codon Plus (DE3)-RIL after the hGHBP cDNA coding sequence for residues 1–238 was ligated into the pET20b(+) vector. Codon Plus cells were grown in 500 ml 2YT medium by shaking at 200 rev min^{-1} at 310 K in 2 l flasks to an A_{600} of 0.8, after which IPTG (1.0 m*M*) was added. The cells were incubated for an additional 3 h at 293 K, harvested, PMSF was added to a final concentration of 0.2 m*M* and the paste was stored frozen at

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203 K overnight. In a modification of the method of Sakal *et al.* (2000), the inclusion-body pellet obtained from 2 l bacterial culture by French Press lysis and washing was solubilized in 400 ml 4.5 *M* urea buffered with 40 m*M* Tris base pH 10.4. L-Cysteine was added to 0.1 m*M* and the clear solution was stirred at 277 K for 48 h. This solution was then dialyzed for 48 h against six changes of 10 m*M* Tris–HCl pH 8.1 at 277 K. The solution was subsequently loaded at 120 ml h^{-1} onto a Q-Sepharose column (1.6 × 20 cm) pre-equilibrated with 10 m*M* Tris–HCl pH 8.1 at 277 K. Elution was carried out with a continuous NaCl gradient (0–0.4 *M*) in the same buffer. Fractions eluting between 0.05 and 0.25 *M* NaCl were analyzed by 12.5% SDS–PAGE in the presence or absence of reducing agents and fractions judged to be greater than 95% purity were pooled.

2.2. Crystallization

Crystallization trials were carried out by the hanging-drop vapour diffusion method at 295 K using crystallization screens described by Jancarik & Kim (1991) and Cudney *et al.* (1994). A 2 µl droplet of concentrated growth hormone receptor extracellular ligand-binding domain (4 mg ml^{-1}) was mixed in a 1:1 ratio with the reservoir solution and equilibrated against 1 ml reservoir solution. Crystals initially took five months to appear in condition No. 26 of Hampton Screen 2 [Hampton Research, California, USA; 100 m*M* MES pH 6.5 containing 30%(*w*/*v*) PEG 5000 monomethyl ether and 200 m*M* ammonium sulfate], growing as small rods. Streak-seeding from these crystals into fresh drops resulted in significantly larger crystals that grew within two weeks. A combination of streak-seeding and optimization of pH and precipitant concentration resulted in crystals that were deemed to be suitable for diffraction studies. The largest crystals were obtained using a reservoir solution consisting of 100 m*M* HEPES pH 7.0 containing 27.5%(*w*/*v*) PEG 5000 monomethyl ether and 200 m*M* ammonium sulfate (Fig. 1).

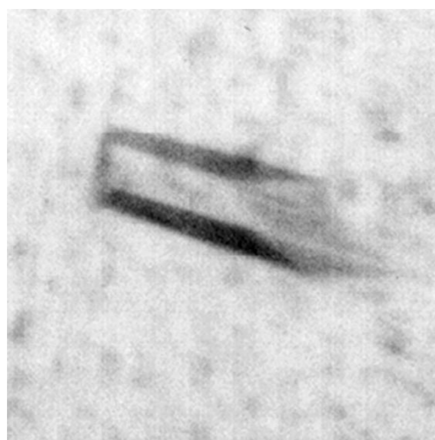

Figure 1
Growth hormone receptor extracellular ligand-binding domain crystals. Dimensions are 0.20 × 0.05 × 0.05 mm.

2.3. Data collection

Prior to data collection, a single crystal was transferred into a stabilizing solution of 100 m*M* HEPES pH 7.0 containing 30%(*w*/*v*) PEG 5000 monomethyl ether and 200 m*M* ammonium sulfate and sealed within a glass capillary for transport at room temperature to the Advanced Photon Source, Chicago, USA. The crystal was soaked in a stabilizing solution containing 5%(*v*/*v*) glycerol for 5 min, flash-cooled to 100 K and exposed to X-rays. An X-ray data set was measured on a MAR Research CCD.165 detector on BioCARS beamline 14-ID-B. The data set extended to 2.5 Å resolution (Fig. 2). The crystals were found to belong to space group $C222_1$, with unit-cell parameters $a = 99.7$, $b = 112.2$, $c = 93.2$ Å. The images were processed and scaled using the *HKL* program package (Otwinowski & Minor, 1997) and the statistics are shown in Table 1. A total of 17 070 unique reflections were measured with an overall redundancy of 35.7. The merged data set is 92.8% complete to 2.5 Å resolution, with an $R_{\rm sym}$ of 8.3% and an $I/\sigma(I)$ of 20.2. The calculated Matthews coefficient ($V_{\rm M}$) of 2.41 Å^3 Da^{-1} suggested the presence of two molecules in the asymmetric unit and a solvent content of about 45% (Matthews, 1968). There was no peak observed in the self-rotation function that would confirm the presence of twofold non-crystallographic symmetry, although we could not exclude the likelihood that dimers of the extracellular ligand-binding domain exist in the crystal. However, biochemical evidence using size-exclusion chromatography suggests that the expressed domain exists as a monomer. Currently, there is debate within the literature as to whether the growth hormone receptor exists as a preformed dimer or undergoes a conformational change in response to growth-hormone binding (Frank, 2002). We plan to solve the crystal structure using the method of molecular replacement based on the published model for the holo-receptor complex (de Vos *et al.*, 1992). The availability of the crystal structure for the unliganded growth hormone receptor's extracellular domain will thus provide some insights into its mode of action..

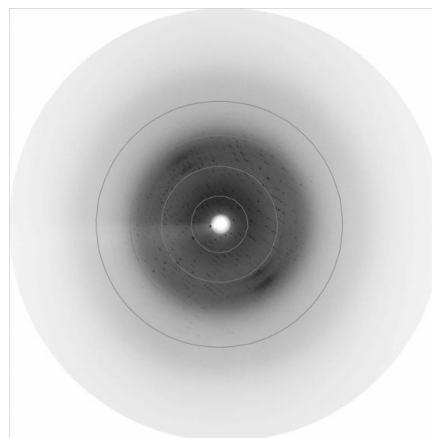

Figure 2
A representative 1° oscillation image of data collected from a crystal of the unliganded human growth hormone receptor extracellular ligand-binding domain, showing Bragg reflections extending beyond 2.5 Å.

Table 1
Crystal data and X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution bin (approximate 0.1 Å interval).

X-ray source	Beamline 14-ID-B, APS (Chicago, USA)
X-ray wavelength (Å)	0.99988
Temperature (K)	100
Space group	$C222_1$
Unit-cell parameters (Å)	$a = 99.7$, $b = 112.2$, $c = 93.2$
Resolution (Å)	2.5
Total observations	609914
Total No. unique reflections	17070
Data completeness (%)	92.8 (52.5)
$R_{\rm sym}$† (%)	8.3 (33.1)
$I/\sigma(I)$	20.2 (3.2)

† $R_{\rm sym} = \sum(I - \langle I \rangle)/\sum(I)$, where I is the intensity measurement for a given reflection and $\langle I \rangle$ is the average intensity for multiple measurements of this reflection.

WJM is supported by a National Health and Medical Research Council of Australia (NHMRC) Industry Fellow, MJW is a NHMRC Principal Research Fellow and MWP is a NHMRC Senior Principal Research Fellow. This work was supported in part from grants from the NHMRC. We thank Dr Harry Tong and other staff at BioCARS for their help with data collection during our visits to the Advance Photon Source. This work, including use of the BioCARS sector, was supported by the Australian Synchrotron Research Program, which is funded by the Commonwealth of Australia under the Major National Research Facilities Program. Use of the Advanced Photon Source was supported by

the US Department of Energy, Basic Energy Sciences, Office of Energy Research.

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