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Crystallization and preliminary X-ray diffraction analysis of human growth and differentiation factor 5 (GDF-5)

Thomas D. Mueller,* Maike Gottermeier, Walter Sebald and Joachim Nickel

Lehrstuhl für Physiologische Chemie II, Theodor-Boveri Institut für Biowissenschaften (Biozentrum) der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

Correspondence e-mail: mueller@biozentrum.uni-wuerzburg.de

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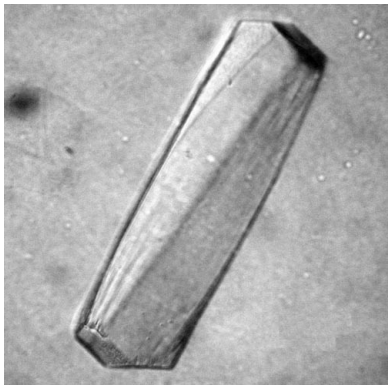

Growth and differentiation factor 5 (GDF-5) belongs to the large TGF-β superfamily of secreted signalling proteins and plays a pivotal role in skeletal development during embryogenesis. The gene for human GDF-5 was cloned, expressed in *Escherichia coli* and purified to homogeneity. Crystals were obtained that diffracted to 2.2 Å resolution. A native data set was acquired, showing that the crystals belong to a trigonal space group, *i.e.* $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 97.1$, $c = 48.3$ Å. Initial analysis suggest the presence of only one monomer in the asymmetric unit, resulting in a high solvent content of 72% in the crystal.

1. Introduction

The bone morphogenetic proteins (BMPs) belong to the large TGF-β superfamily of secreted signalling proteins. They play important roles in skeletal development and are capable of inducing ectopic bone formation *in vivo* by an endochondral pathway (Yoon & Lyons, 2004). This includes the recruitment of progenitor cells followed by a cellular condensation to form cartilaginous precursors and the replacement of cartilage by bone (Rosen & Thies, 1992). Several BMPs are expressed at sites of bone formation with a defined spatiotemporal pattern, which indicates that a coordinated expression pattern is crucial for endochondral ossification (Lyons *et al.*, 1990). Growth and differentiation factor-5 (GDF-5) belongs together with GDF-6 and GDF-7 to a subfamily of the BMPs. GDF-5 has been reported to affect multiple skeletal processes including joint formation (Francis-West *et al.*, 1999; Settle *et al.*, 2003; Storm & Kingsley, 1999) and tendon repair (Aspenberg & Forslund, 1999; Wolfman *et al.*, 1997). Naturally occurring mutations in the GDF-5 gene in both humans and mice result in diseases such as brachypodism and chondrodysplasia (Polinkovsky *et al.*, 1997; Storm & Kingsley, 1996; Thomas *et al.*, 1996).

This paper describes the crystallization and preliminary X-ray crystallographic studies of GDF-5. Three-dimensional structural studies of GDF-5 in comparison to crystal structures of other TGF-β superfamily members are expected to elucidate the molecular basis for the binding affinity and specificity in BMP/GDF-receptor recognition. Knowledge of how specificity is generated in the TGF-β superfamily is crucial for the design of highly receptor-specific agonists and antagonists that present useful therapeutics in GDF-5-associated diseases (Mikic, 2004).

2. Materials and methods

2.1. Protein preparation and expression

Poly(A)-rich RNA was obtained from the human osteosarcoma cell line U2OS (ATCC HTB-96) and reverse-transcribed into cDNA using standard techniques. The cDNA encoding amino acids 387–501 of hGDF-5 plus an N-terminal MK extension was amplified by PCR and cloned into the *Nco*I/*Bam*HI sites of the expression vector $RBSII_{N25}x/o$ (Stueber *et al.*, 1984). For protein expression, the expression plasmid $RBSII_{N25}x/o$-GDF5 was transformed into *Escherichia coli* strain JM109 host cells. Transformed cells were grown in LB medium at 310 K until the optical density at 600 nm reached 0.7. Protein overexpression was induced by the addition of

IPTG (to a final concentration of 1 m*M*). Human GDF-5 was expressed in inclusion bodies; refolding of GDF-5 followed the protocol published for BMP-2 (Ruppert *et al.*, 1996). After refolding, native dimeric GDF-5 was separated from inactive monomeric protein by cation-exchange chromatography using Fractogel EMD-COO^- (Merck) employing an NaCl gradient of 0–1.5 *M* in 50 m*M* sodium acetate pH 5.0, 30%(*v*/*v*) 2-propanol. Pure protein was dialyzed extensively against water and freeze-dried for storage. Purity and homogeneity was assessed by RP-HPLC and FT-ICR mass spectroscopy.

2.2. Crystallization

For crystallization, GDF-5 was dissolved in water or 0.1 m*M* HCl to a final concentration of 2.5–10 mg ml^{-1}. Initial crystallization experiments were performed by hanging-drop vapour-diffusion and sparse-matrix screening methods (Cudney *et al.*, 1994; Jancarik & Kim, 1991) using tissue-culture multiwell plates with covers (XRL, Molecular Dimensions, UK) at a temperature of 294 K. Each hanging drop was prepared by mixing 1 µl each of protein solution and reservoir solution and was placed over 500 µl reservoir solution. Initial conditions were screened using Crystal Screen I and II (Hampton Research, USA) and JBScreen 7 and 8 (Jena Bioscience, Germany) kits. Crystal optimization was carried out by altering the concentrations of the salt, precipitant and protein and the ratio between protein and reservoir solution in the hanging drop, as well as the pH of the GDF-5 protein solution used for setting up the crystallization trials.

2.3. X-ray data collection

A complete data set was collected from a single crystal at beamline X06SA of the Swiss Light Source (SLS, Paul Scherrer Institute, Switzerland). Crystals were mounted in a nylon loop and flash-frozen in liquid nitrogen without further soaking. The crystal-to-detector distance was set to 190 mm, the wavelength was 0.918 Å and all data collection was performed at 100 K. For high-resolution data the crystal was rotated through a total of 90°, with a 1° oscillation per frame and an exposure time of 30 s per frame. Low-resolution data were acquired using the same setup and oscillation range, but the exposure time was set to 1 s per frame. Data processing was performed using the software *MOSFLM* (Leslie, 1992) and *SCALA* (Evans, 1993). Data-collection and processing statistics are given in Table 1.

Table 1
Data-collection and processing statistics.

Values in parentheses correspond to the highest resolution shell.

Space group	$P3_121$ (or $P3_221$)
Unit-cell parameters (Å, °)	$a = b = 97.1$, $c = 48.3$, $\alpha = \beta = 90$, $\gamma = 120$
Matthews coefficient† (Å^3 Da^{-1})	4.38
Resolution (Å)	17.0–2.28 (2.39–2.28)
Total No. reflections	54932 (5079)
No. unique reflections	10907 (1346)
Completeness	96.4 (95.3)
Redundancy	5.1 (3.8)
$R_{\rm merge}$‡ for all reflections (%)	5.9 (19.4)
$R_{\rm merge}$‡ for full reflections only (%)	5.3 (13.4)
$\langle I/\sigma(I)\rangle$	8.1 (3.4)

† Assuming one monomer in the asymmetric unit. ‡ $R_{\rm merge} = \sum|I_j - \langle I_j\rangle|/\sum\langle I_j\rangle$, where I_j is the intensity of reflection j and $\langle I_j\rangle$ is the average intensity of reflection j.

3. Results and discussion

Initial crystals could be obtained from several crystallization conditions containing alcohols, *e.g.* 2-propanol and 2-methyl-2,4-pentanediol (MPD), or polyethyleneglycols (molecular-weight range 2000–4000) at various pH values from acidic to basic. Crystals grown from 20–35%(*v*/*v*) MPD and at acidic pH (sodium citrate pH 4.5) exhibited a single-crystal morphology and diffracted to high resolution (Fig. 1). For data acquisition, crystals were grown at 294 K from 25%(*v*/*v*) MPD, 0.1 *M* sodium citrate pH 4.0; the protein concentration was 2.4 mg ml^{-1} and the ratio of protein to reservoir solution was 2:1. Crystals grew reproducibly within 5 d to approximate dimensions of 0.5 × 0.1 × 0.1 mm and diffracted to 2.2 Å. Fig. 2 shows a typical X-ray diffraction pattern of such a crystal. A complete data set consisting of 10 907 unique reflections was merged from a low- and a high-resolution run with adjusted exposure times to avoid incomplete

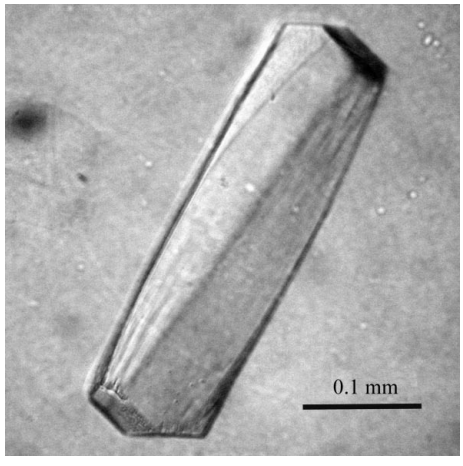

Figure 1
Crystals of human GDF-5 grown in 25% MPD and 0.1 *M* sodium citrate pH 4.0 using the hanging-drop method. Dimensions of the crystal are approximately 0.4 × 0.07 × 0.07 mm.

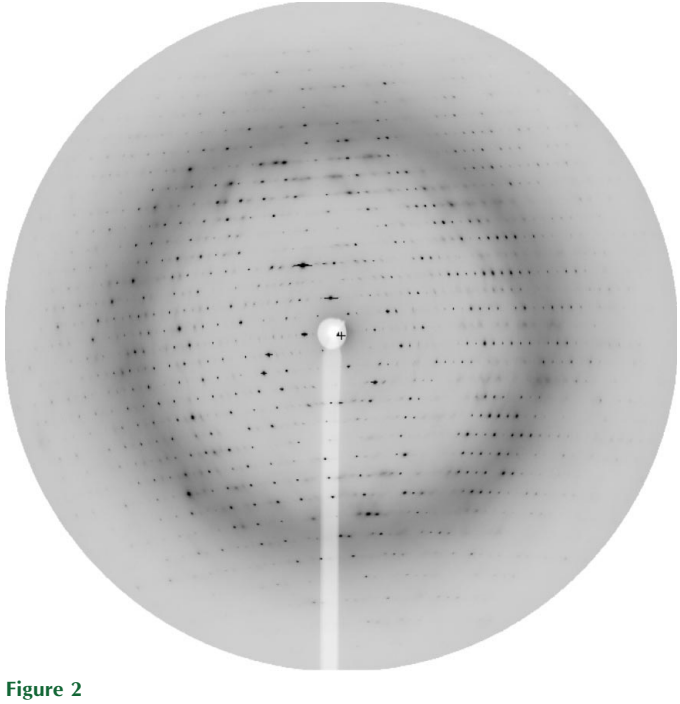

Figure 2
X-ray diffraction pattern obtained for the crystal in Fig. 1; the crystal-to-detector distance was 190 mm and the resolution limit at the edge of the diffraction image is 2.25 Å.

data arising from overload. The overall $R_{\rm merge}$ was 5.9% and the completeness was 96.4%. The crystal belongs to the trigonal space group $P3_121$ (or $P3_221$), with unit-cell parameters $a = b = 97.1$, $c = 48.3$ Å. Assuming that the asymmetric unit contains one monomer subunit of the native dimeric GDF-5 (molecular weight 13 384 Da), the $V_{\rm M}$ value is 4.38 Å^3 Da^{-1}; for a dimer in the asymmetric unit the $V_{\rm M}$ value is 2.19 Å^3 Da^{-1}. The corresponding solvent content of the crystal is calculated to be 72% for one monomer per asymmetric unit and 44% if two monomers are present in the asymmetric unit. A native Patterson map did not reveal any non-crystallographic twofold symmetry, suggesting that only one monomer is present in the asymmetric unit and indicating a high solvent content in these crystals. With one exception (Mittl *et al.*, 1996), a similar high solvent content of more than 65% has also been observed in crystals of other members of the TGF-β superfamily (usually 60–75%; Daopin *et al.*, 1992; Griffith *et al.*, 1996; Mittl *et al.*, 1996; Scheufler *et al.*, 1999; Schlunegger *et al.*, 1992), suggesting that crystal packing for proteins of this superfamily is not very tight. Molecular-replacement methods will be used to solve the structure of human GDF-5.

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