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Crystallization and preliminary X-ray crystallographic analysis of osteoclast-stimulating factor

Ming Li,[a,b] Zhaohui Meng,[a,b] Yanhui Xu,[a,b] Zhiyong Lou[a,b] and Zihe Rao[a,b]*

[a]Laboratory of Structural Biology, Tsinghua University, Beijing 100084, People's Republic of China, and [b]National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Science, Beijing 100101, People's Republic of China

Correspondence e-mail: raozh@xtal.tsinghua.edu.cn

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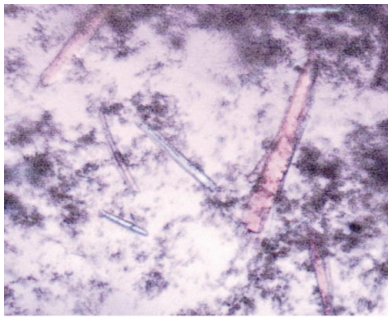

Osteoclast-stimulating factor increases osteoclast formation and bone resorption through a cellular signal transduction cascade, possibly by its interaction with c-Src or related family members. Crystals of human osteoclast-stimulating factor were obtained by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. The crystals are primitive orthorhombic and belong to *P*222 or a related space group, with unit-cell parameters a = 38.1, b = 54.9, c = 64.7 Å.

1. Introduction

The osteoclast is the primary bone-resorbing cell and factors produced by the osteoclast itself play an important role in regulating osteoclast formation and activity (Roodman, 1996; Reddy & Roodman, 1998). One novel intracellular protein produced by osteoclasts, termed osteroclast-stimulating factor (OSF), has been isolated and identified. This 28 kDa peptide, containing a c-Src homology 3 domain (SH3) and ankyrin repeats (Reddy *et al.*, 1998), could indirectly induce osteoclast formation and bone resorption. OSF can bind c-Src *in vitro*, which suggests a possible role for OSF in the bone-resorption process through protein interactions with the cellular signal transduction machinery. Ankyrin repeats have also been confined to specific membrane domains and bind integral membrane proteins such as the (Na–K)-ATPase and the voltage-dependent Na channel in haematopoietic cells (Lux *et al.*, 1990). A spinal muscular atrophy disease-determining gene product, survival motor neuron (SMN), may interact with the OSF-Src homology 3 domain (Kurihara *et al.*, 2001). This interaction may provide further insight into novel cellular signalling mechanisms that may play an important role in the congenital bone fractures associated with type I spinal muscular atrophy disease. A mouse homologue, SH3P2, a novel Cbl-interacting molecule, is a substrate of tyrosine kinase Src, which leads to increased tyrosine phosphorylation of SH3P2 (Szymkiewicz *et al.*, 2004). This indicates that OSF might be involved in Cbl- and Src-mediated pathways.

The gene encoding OSF (OSTF1) was localized to human chromosome band 12q24.1 (Schaub *et al.*, 2000). OSF mRNA (1.3 kbp) is ubiquitously expressed in multiple human tissues and encodes a 28 kDa peptide. In this report, we describe the crystallization and preliminary X-ray diffraction studies of this protein.

2. Materials and methods

2.1. Expression and purification

The primers used for OTSF1 were OSF F, 5′-GAATTCATGTC-GAAGCCGCCACCCAA-3′, and OSF R, 5′-CCGCTCGAGTTA-ATCTGAGTCTA-3′. OTSF1 was amplified by PCR, cloned into pGEX-6p-1 (Pharmacia) and transferred into *Escherichia coli* strain BL21(DE3). Transformed cells were grown at 310 K in 2×YT medium with 100 µg ml^{-1} ampicillin added to an optical density of 0.8–1.0 (OD_{600}) before induction with 0.1 m*M* IPTG for 4 h at 289 K. Bacterial cells were harvested by centrifugation at 5000*g* for 10 min at 277 K. Following harvesting, the cell pellets were resuspended in phosphate-buffered saline (PBS) and the cells were disrupted by

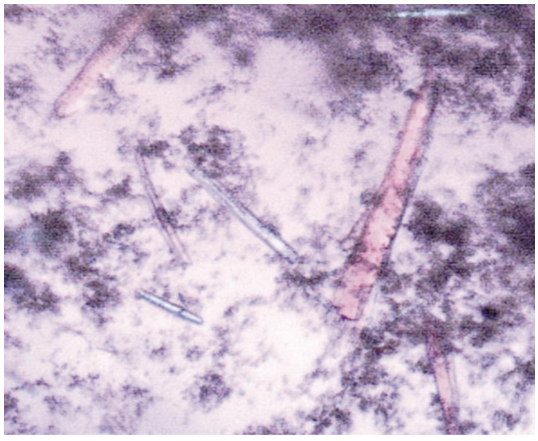

Figure 1
Typical crystals of OSF in 0.1 *M* Tris–HCl pH 8.0 containing 2.4 *M* $(NH_4)_2SO_4$. The length of the crystals is about 3 mm.

sonicating 200 times for 4 s periods with 6 s intervals. Cell debris was removed by centrifugation of the lysates at 15 000*g* for 30 min at 277 K.

All the following purification steps were performed at 289 K. The clear supernatant was loaded onto a Glutathione Sepharose 4B column (Pharmacia) equilibrated with PBS and the contaminated protein was then eluted using PBS. The GST tag was cleaved by PreScission protease overnight at 277 K and then the target protein was further eluted with PBS. Further purification was achieved by gel-filtration chromatography on Superdex 75 (Pharmacia) with 0.1 *M* Tris–HCl pH 8.0 containing 0.15 *M* NaCl and analyzed by SDS–PAGE.

2.2. Crystallization

The purified protein was dialyzed against crystallization buffer (10 m*M* Tris–HCl pH 8.0 containing 10 m*M* NaCl) and concentrated to 10–15 mg ml^{-1}. Protein concentrations were determined by absorbance at 280 nm, assuming an A_{280} of 1.555 for a 1.0 mg ml^{-1} solution. Preliminary crystallization experiments were carried out at 291 K in 16-well plates with Crystal Screen reagent kits I and II (Hampton Research) using the hanging-drop vapour-diffusion method (Jancarik & Kim, 1991). Drops containing 1 µl protein solution and an equivalent volume of reservoir solution were equilibrated against 200 µl reservoir solution. The protein sample was centrifuged at 13 000 rev min^{-1} for 13 min to clarify the solution before initiating any crystal trials. The conditions yielding optimum crystals were 0.1 *M* Tris–HCl pH 8.0 containing 2.4 *M* $(NH_4)_2SO_4$. The crystals appeared after two months.

2.3. Data collection and processing

Data collection of OSF was performed in-house on a Rigaku RU2000 rotating copper-anode X-ray generator operated at 48 kV and 98 mA (Cu $K\alpha$; λ = 1.5418 Å) with a MAR 345 image-plate detector. Crystals were picked up from the hanging drop with a nylon crystal loop (Hampton Research) and then flash-frozen prior to data collection at 100 K. Data processing was performed using the program *DENZO* and data sets were scaled and merged using *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Data-collection and processing statistics of OSF.

Values in parentheses are for the highest resolution shell.

Space group	*P*222 or related
Unit-cell parameters (Å)	a = 38.1, b = 54.9, c = 64.7
Wavelength (Å)	1.5418
Resolution range (Å)	50.0–2.6
Observed reflections	19958
Unique reflections	5030
Completeness (%)	99.0 (100.0)
$I/\sigma(I)$	7.5 (2.2)
R_{sym} (%)	13.9 (41.5)

3. Results and discussion

The molecular weight of the protein was found to be 20 kDa, which was lower than expected. The nucleotide sequence of the plasmid was checked and it was found that residues 163–195 of OSF were missing. The purity of the final protein was confirmed to be greater than 90% by SDS–PAGE analysis and judged to be suitable for use in crystallization trials. The optimum quality crystals were obtained from a condition containing 0.1 *M* Tris–HCl pH 8.0 with 2.4 *M* $(NH_4)_2SO_4$ over a period of two months. The crystals were suitable for X-ray diffraction (Fig. 1).

X-ray diffraction data were collected from a single crystal of OSF using a Rigaku RU2000 rotating copper-anode X-ray generator and a MAR 345 image-plate detector. The crystal-to-detector distance was 120 mm, the exposure time was approximately 600 s, the oscillation angle was 1° and the wavelength was 1.5418 Å. The OSF crystals are primitive orthorhombic and belong to *P*222 or a related space group, with unit-cell parameters a = 38.1, b = 54.9, c = 64.7 Å, α = 90, β = 90, γ = 90° (Figs. 2*a* and 2*b*). Assuming the presence of one molecule in the asymmetric unit and a molecular weight of 20 kDa, the solvent content is calculated to be about 26.8% and the Matthews coefficient (V_M) is about 1.7 Å^3 Da^{-1}. Selected data statistics are shown in Table 1. Structure determination of OSF is currently under way.

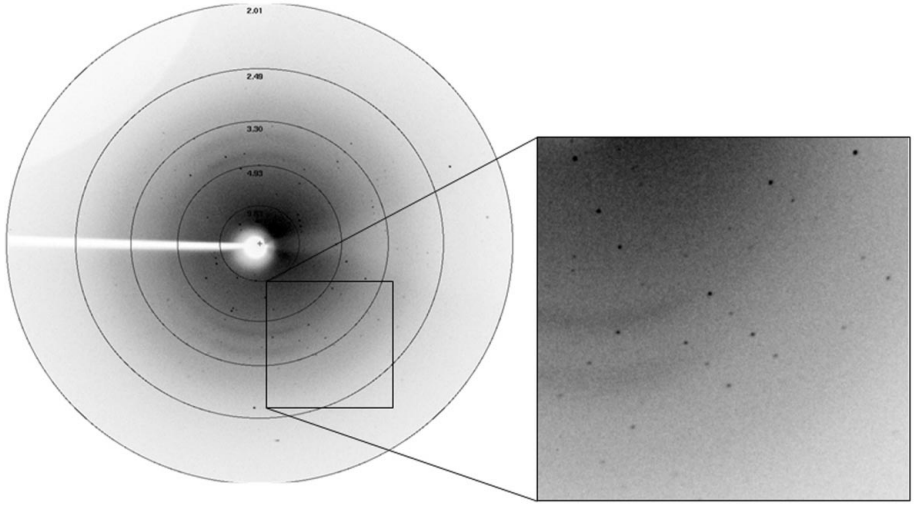

Figure 2
(*a*) A typical X-ray diffraction pattern from a crystal of OSF. The diffraction image was collected on a MAR Research image-plate detector. The oscillation range is 1°. Resolution rings correspond to 9.83, 4.93, 3.30, 2.49 and 2.01 Å. (*b*) An enlarged image of the area indicated in (*a*).

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