# Structure of rat acidic fibroblast growth factor at 1.4 Å resolution

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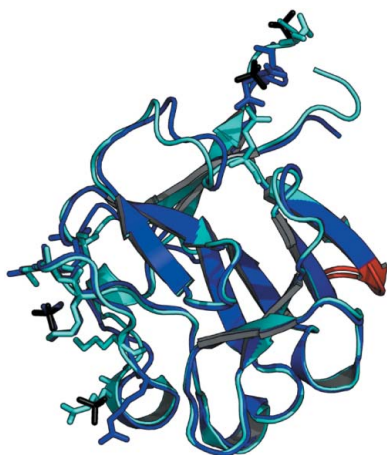
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Fibroblast growth factors (FGFs) constitute a family of 22 structurally related heparin-binding polypeptides that are involved in the regulation of cell growth, survival, differentiation and migration. Here, a 1.4 Å resolution X-ray structure of rat FGF1 is presented. Two molecules are present in the asymmetric unit of the crystal and they coordinate a total of five sulfate ions. The structures of human, bovine and newt FGF1 have been published previously. Human and rat FGF1 are found to have very similar structures.

## 1. Introduction

Fibroblast growth factors (FGFs) constitute a large family of signalling molecules that play important roles in physiological processes such as embryogenesis, cell differentiation, growth, angiogenesis, tissue repair and wound healing (Ornitz & Itoh, 2001). When mutated or inappropriately expressed, FGFs can cause various pathological processes resulting in morphogenetic disorders and cancer. Different FGFs demonstrate different expression patterns, ranging from nearly ubiquitous expression in the case of FGF2 to highly restricted expression in the case of FGF4 (Basilico & Moscatelli, 1992). FGFs signal by activating their specific cell-surface receptors (FGFRs) encoded by four distinct genes (FGFR1–4), which can produce numerous FGFR isoforms through alternative splicing (Johnson & Williams, 1993; Schlessinger, 2000). FGFRs are transmembrane proteins with tyrosine kinase activity. Ligand binding to the extracellular domain of the receptor activates signalling cascades, resulting in modification of gene expression. One receptor can bind and become activated by several FGF ligands (Ornitz *et al.*, 1996). FGF–FGFR interaction also requires the participation of heparin or heparan sulfate proteoglycans (HSPGs), which bind both FGF and FGFR, stabilizing the formation of a receptor dimer (Yayon *et al.*, 1991; Schlessinger *et al.*, 2000). Thus, the activity of FGFs is regulated at multiple levels, including growth-factor expression, receptor-binding affinity and interaction with HSPG.

All FGFs share the $\beta$-trefoil fold first described for the soybean trypsin inhibitor (Sweet *et al.*, 1974). The structural architecture of the fold consists of 12 $\beta$-strands that form six $\beta$-hairpins. The name of the fold originates from the threefold symmetry, which produces three 'trefoil' subdomains of four $\beta$-strands each (Sweet *et al.*, 1974; Bernett *et al.*, 2004). Other proteins that share this structural fold include interleukin-1$\alpha$ and interleukin-1$\beta$ (Priestle *et al.*, 1989), plant cytotoxins (Rutenber *et al.*, 1991), bacterial toxins (Lacy *et al.*, 1998), an actin-binding protein (Habazettl *et al.*, 1992), amylase (Vallee *et al.*, 1998), xylanase (Kaneko *et al.*, 1999) and mannose receptor (Liu *et al.*, 2000).

Structures of human (Brych *et al.*, 2001; Bernett *et al.*, 2004), bovine (Zhu *et al.*, 1991) and newt FGF1 (PDB code 1fmm) have been reported previously. Here, we report the crystal structure of rat FGF1 at 1.4 Å. The structure was compared with those of human, bovine and newt FGF1.

## 2. Experimental

A cDNA fragment encoding rat FGF1 (residues 22–155; Swiss-Prot No. P61149) was synthesized by PCR using Quick-Clone Rat Brain cDNA (BD Biosiences) from 8–12-week-old Sprague–Dawley rats. The amplified cDNA fragment was subcloned into the *Eco*RI/*Hin*dIII cloning site of pQE-60 plasmid (Qiagen). *Escherichia coli* strain Top10F′ (Invitrogen) was used for transformation. The expression of the protein was induced by adding isopropyl $\beta$-D-thiogalacto-pyranoside (IPTG; Sigma). The protein was purified by affinity chromatography using a 5 ml HiTrap Heparin HP column (GE Healthcare). Finally, gel filtration was performed in phosphate-buffered saline (PBS, Sigma) using a Superdex 75 column (GE Healthcare).

Crystallization of the protein was carried out in hanging-drop vapour-diffusion experiments by mixing 1 µl protein (10 mg ml$^{-1}$) and 1 µl reservoir solution (1.6 M ammonium sulfate, 0.1 M citric acid pH 5.0). Large crystals were obtained at room temperature within 4 d (Fig. 1).

X-ray data were collected at 120 K from a cryoprotected crystal (crystallization solution containing 20% glycerol) using synchrotron radiation (ID23-1, ESRF). The data were indexed, integrated and scaled using the programs *MOSFLM* and *SCALA* (Collaborative Computational Project, Number 4, 1994). All statistics are listed in Table 1.

The FGF1 structure was determined by the molecular-replacement method using the program *Phaser* (Storoni *et al.*, 2004). A crystal structure of bovine FGF1 (PDB code 1afc; Zhu *et al.*, 1993) was used as the search model and solutions accounting for the two molecules in the asymmetric unit were obtained. Subsequently, automated model building was performed using the program *ARP/wARP* (Perrakis *et al.*, 1999). This resulted in the tracing of 92% of the residues. The missing residues were inserted manually using the program *Coot* (Emsley & Cowtan, 2004). Structure refinement was performed in the program *REFMAC*5 (Murshudov *et al.*, 1999). Water molecules and five sulfate ions were gradually introduced into the structure. The quality of the structure was evaluated using the program *PROCHECK* (Laskowski *et al.*, 1993). Figures were prepared using the program *PyMOL* (DeLano, 2002).

## 3. Results and discussion

The structure was refined at 1.4 Å (see Table 1 for details). The atomic structure of FGF1 consists of two molecules in the asymmetric
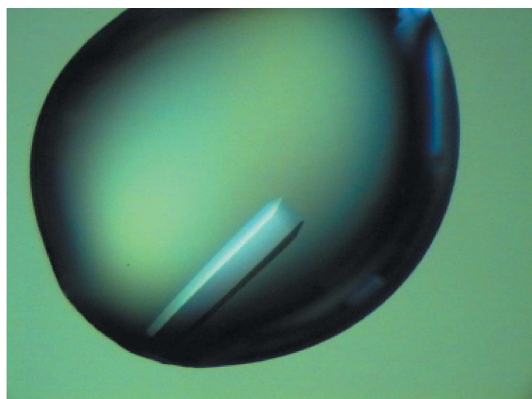


### Figure 1
Crystal of rat FGF1 belonging to space group $P2_12_12_1$. The crystal dimensions are ~500 × 100 × 100 Å.

### Table 1
Data-collection and processing statistics for FGF1.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| X-ray source | ID23-1, ESRF, Grenoble |
| Wavelength (Å) | 0.9792 |
| Space group | $P2_12_12_1$ |
| Unit-cell parameters | |
|   $a$ (Å) | 42.9 |
|   $b$ (Å) | 61.7 |
|   $c$ (Å) | 88.9 |
| Mosaicity (°) | 0.55 |
| Resolution (Å) | 50–1.4 (1.48–1.4) |
| No. of observations | 353617 |
| No. of unique reflections | 47203 |
| Redundancy | 7.5 |
| Completeness (%) | 99.9 (99.9) |
| $I/\sigma(I)$ | 4.3 (3.3) |
| $R_{merge}$† (%) | 7.7 (20.9) |
| $V_M$ (Å$^3$ Da$^{-1}$) | 1.8 |
| Refinement | |
|   Protein atoms | 2197 |
|   Protein residues | |
|     Chain $A$ | 22–155 |
|     Chain $B$ | 24–155 |
|   Other atoms | 258 water molecules, 5 sulfate ions (25 atoms) |
|   $R_{conv}$‡ (%) | 20.4 |
|   $R_{free}$§ (%) | 22.9 |
|   Mean $B$ values (Å$^2$) | |
|     Molecule $A$ | |
|       Main chain | 10.8 |
|       Side chain | 13.1 |
|     Molecule $B$ | |
|       Main chain | 10.1 |
|       Side chain | 12.1 |
|     Water atoms | 23.0 |
|     Sulfate atoms | 26.5 |
|   R.m.s.d. bond lengths (Å) | 0.008 |
|   R.m.s.d. bond angles (°) | 1.2 |

† $R_{merge} = \sum_{hkl}[\sum_i(|I_{hkl,i} - \langle I_{hkl}\rangle|)]/\sum_{hkl,i}\langle I_{hkl}\rangle$, where $I_{hkl,i}$ is the intensity of an individual measurement of the reflection with Miller indices $hkl$ and $\langle I_{hkl}\rangle$ is the mean intensity of that reflection. ‡ $R_{cryst} = \sum_{hkl}||F_{o,hkl}| - |F_{c,hkl}||/|F_{o,hkl}|$, where $|F_{o,hkl}|$ and $|F_{c,hkl}|$ are the observed and calculated structure-factor amplitudes, respectively. § $R_{free}$ is equivalent to the $R$ factor, but is calculated with reflections omitted from the refinement process (5% of reflections were omitted).

unit (molecules $A$ and $B$; see Fig. 2$a$), five sulfate ions and 258 water molecules. The root-mean-square deviation (r.m.s.d.) on C$^\alpha$ atoms 26–152 (omitting obviously disordered residues from the N-terminus) of the $A$ and $B$ molecules is 0.76 Å. The Ramanchandran plot showed 93.3% of the residues to be in the most favoured region, 6.2% in the additionally allowed region and 0.4% in the generously allowed region. Apart from the differences in the N- and C-terminal regions, conformational differences are seen in the loop region harbouring Ala66 (residues 64–67; see Fig. 2$a$). This is probably related to the larger number of hydrophobic contacts to the crystal environment of the $B$64–$B$67 loop, where 16 distances shorter than 4 Å are observed as opposed to only eight in the $A$64–$A$67 loop. Furthermore, molecule $A$ has marginally higher $B$ values ($B_{avg}$ = 13.1 Å$^2$) than molecule $B$ ($B_{avg}$ = 12.1 Å$^2$).

Five ordered sulfate ions are coordinated to the FGF1 molecules (see Fig. 2$a$). Three of the ions are bound to the high-affinity heparin-binding site at a position similar to that where sucrose octasulfate (Zhu *et al.*, 1993) or heparin (DiGabriele *et al.*, 1998) bind in human FGF1. The fourth and fifth sulfate ions are bound to FGF1 residues (Ser153, Ser154 and Asp155) that have not been demonstrated to be involved in heparin binding.

The rat FGF1 structure (molecule $A$) was compared with those of human (molecule $A$), bovine (molecule $A$) and newt (molecule 1) origin. As expected based on the 96.4% sequence identity, the structure of rat FGF1 is very similar to the structure of human FGF1, with an r.m.s.d. of 0.68 Å on 127 C$^\alpha$ atoms (see Fig. 2$b$). Similar
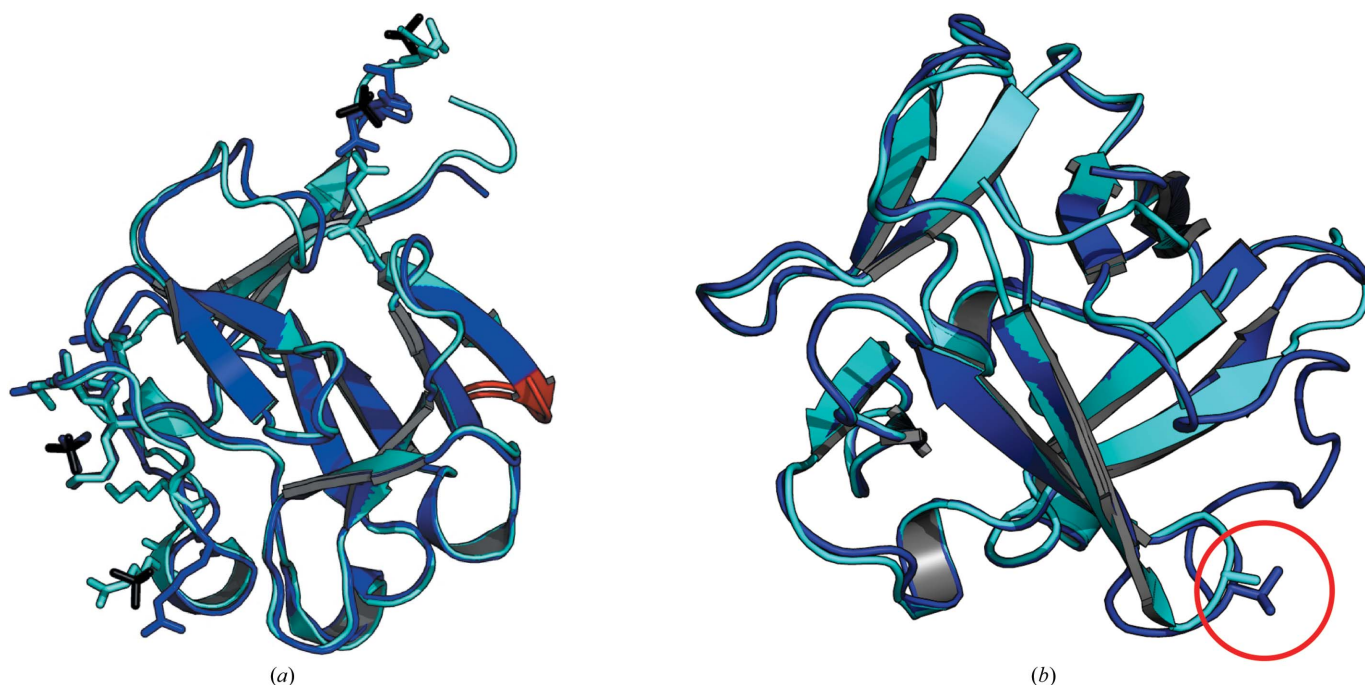
**Figure 2**
Structure of FGF1. (*a*) Superimposition of molecules *A* and *B* of rat FGF1 (coloured blue and cyan). The five sulfate ions present in the asymmetric unit of the crystal are coloured black. Loop Glu64–Gly67 is coloured red. The amino acids involved in binding of sulfate ions are Asn33, Lys127, Lys128, Lys133, Arg134, Arg137, Ser153, Ser154 and Asp155. (*b*) Superimposition of human (blue) and rat (cyan) FGF1s. The position of the amino-acid substitution (Val66, human; Ala66, rat) in the FGFR-interacting region is marked with a circle.

structural variations were observed when the rat structure was compared with the less closely related 2.7 Å structure of bovine FGF1 (the sequence identity between rat and bovine FGF1 is 90.7%), showing an r.m.s.d. value of 0.64 Å (127 $C^\alpha$ atoms). Lager structural variations were observed when the rat structure was compared with the NMR structure of newt FGF1 (the sequence identity between rat and newt FGF1 is 74.3%), showing an r.m.s.d. value of 2.33 Å (127 $C^\alpha$ atoms).

The alignment of human and rat FGF1 demonstrates only one difference between the amino acids involved in FGFR interaction (human Val66 is substituted by Ala; Mohammadi *et al.*, 2005; Pellegrini *et al.*, 2000; Plotnikov *et al.*, 2000; see Fig. 2*b*). Val66 is the only amino acid involved in spliceform-specific FGF1–FGFR2 Ig module 3 interaction in human (Pellegrini *et al.*, 2000). Among the residues involved in FGF1–heparin interaction, there is no difference between rat and human FGF1 (Nagendra *et al.*, 2001), indicating that the role of heparin in FGF1 signalling is the same in human and rat.

In this communication, we have presented the X-ray crystallographic structure of rat FGF1. The rat FGF1 structure adds to the solid experimental basis of structural insights into FGF1 in mammals and confirms that FGF1 appears to allow only limited structural variation, particularly in functionally important regions of the molecule.

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