Dimerization effect of sucrose octasulfate on rat FGF1.
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Dimerization effect of sucrose octasulfate on rat FGF1

Fibroblast growth factors (FGFs) constitute a family of at least 23 structurally related heparin-binding proteins that are involved in regulation of cell growth, survival, differentiation and migration. Sucrose octasulfate (SOS), a chemical analogue of heparin, has been demonstrated to activate FGF signalling pathways. The structure of rat FGF1 crystallized in the presence of SOS has been determined at 2.2 Å resolution. SOS-mediated dimerization of FGF1 was observed, which was further supported by gel-filtration experiments. The major contributors to the sulfate-binding sites in rat FGF1 are Lys113, Lys118, Arg122 and Lys128. An arginine at position 116 is a consensus residue in mammalian FGF molecules; however, it is a serine in rat FGF1. This difference may be important for SOS-mediated FGF1 dimerization in rat.

1. Introduction

Fibroblast growth factors (FGFs) constitute a large family of signaling molecules that are involved in developmental processes, angiogenesis and tumour growth. At the cellular level, FGFs play important roles in cell differentiation, proliferation, migration and survival (Powers et al., 2000; Ornitz & Itoh, 2001). The expression of different FGFs varies from ubiquitous for FGF2 to highly restricted for FGF4 (Basilico & Moscatelli, 1992). Mutations in FGFs or inappropriate protein expression are correlated with various pathological processes resulting in morphogenetic disorders and cancer (Takahashi et al., 1992; Kornmann et al., 1997). FGFs signal by activating their specific cell-surface receptors (FGFRs). Vertebrates have up to five FGFR paralogues (Powers et al., 2000; Sleeman et al., 2001). The diverse activities of FGFs are mediated by four receptor tyrosine kinases (FGFR1–FGFR4), each composed of an extracellular ligand-binding domain consisting of three immunoglobulin-like modules (Ig1–Ig3), a single transmembrane helix and a cytoplasmic part with protein tyrosine kinase activity (Ornitz et al., 1996). For FGFRs 1–3, alternative splicing of the Ig3 domain results in two isoforms (IIIb and IIIc) with different ligand specificities. One receptor can bind and become activated by several FGF ligands.

Receptor dimerization and binding of heparin or heparan sulfate (HS) proteoglycans (HSPGs) are required for FGF signalling (Yayon et al., 1991; Schlessinger, 2000). HS is produced in cells by modification of the initially synthesized polysaccharide backbone composed of N-acetyl glucosamine and glucuronic acid disaccharide units (Sugahara & Kitagawa, 2002). HS contains 40–300 saccharide units that are modified by a range of enzymes, replacing N-acetyl with N-sulfate, epimerizing glucuronic acid to iduronic acid and adding sulfate groups to iduronic acid or sulfate groups to the O6 glucosamine position. The final versions of HS usually consist of regions of up to 12–14 saccharide units with heavy modifications (NS domains), separated by stretches of 14–18 saccharide units with low levels of modification (S domains). Heparin is a mast-cell-derived analogue of HS that is more highly sulfated but with less complex sulfation patterns. Heparin can replace HS in FGF cellular signalling when HS has been removed by chlorate treatment. Hence, heparin has been
used for indirect studies of the interactions of FGFs with HS (Ormitz et al., 1992; Walker et al., 1994). Variations in the distribution of basic amino acids on the surface of FGFs result in FGF specificity towards different HS sulfate groups (Raman et al., 2003).

Two contrasting models, symmetric and asymmetric, for FGFR dimerization have been proposed based on the crystal structures of FGF–FGFR–heparin complexes. In the symmetric model, two 1:1 FGF2–FGFR1–heparin complexes form a symmetric dimer (Plotnikov et al., 1999; Ponting & Russell, 2000; Schlessinger et al., 2000). Each FGF molecule is monomeric and binds to both receptor molecules in the dimer; direct contacts occur between the two FGFRs. In the complex, heparin binds to both the two FGFs and the two FGFRs, thereby enhancing FGF–FGFR affinity. In the asymmetric model derived from the FGF1–FGFR2–heparin structure, a single heparin oligosaccharide bridges two FGF molecules (forming a FGF–heparin–FGF complex with direct FGF–FGF interactions) that in turn facilitate dimerization of the receptor (Pellegrini et al., 2000).

Heparin binds only to one receptor molecule, resulting in asymmetry of the dimer. Unlike the configuration in the symmetric model, each FGF molecule contacts a single molecule of the receptor and no direct FGFR–FGFR contacts are observed. The total lack of a protein–protein interface between the two FGF–FGFR dimer complexes makes heparin necessary for receptor dimerization in the asymmetric model.

In addition to heparin, a number of chemically diverse low-molecular-weight and sulfated saccharides, such as sucrose octasulfate (SOS), have been reported to potentiate FGF activity. SOS has been shown to mimic heparin action in supporting FGF-induced neoangiogenesis and cell proliferation in vitro (Folkman et al., 1991; Arunkumar et al., 2002; Loughman et al., 1996; Yeh et al., 2002). Moreover, SOS facilitates wound healing by enhancing FGF-induced angiogenesis (Rashid et al., 1999). The molecular mechanism by which SOS stimulates FGF signalling is not fully understood. It has been demonstrated that SOS induces FGF–FGFR dimerization in vitro. The crystal structure of the human dimeric FGF–FGFR–SOS complex has also been published (Yeh et al., 2002) and analysis of this dimeric structure reveals that SOS induces FGF–FGFR dimerization in a similar manner to the symmetric model. In accordance, the bovine FGF1–SOS crystal structure shows the presence of only one SOS molecule bound to the high-affinity heparin-binding site of one FGF1 molecule and thus does not indicate SOS-mediated FGF dimerization (Zhu et al., 1993).

Here, we present the gel-filtration analysis and the crystal structure of rat FGF1 in complex with SOS, showing that rat FGF1 forms a SOS-mediated dimer in solution as well as in crystals. The interactions of SOS with FGF1 have been characterized in protein complexes, revealing new features of SOS binding.

2. Materials and methods

A cDNA fragment encoding rat FGF1 (residues 22–155; Swiss-Prot P61149) was synthesized by polymerase chain reaction using QuickClone Rat Brain cDNA (BD Biosciences). The amplified cDNA fragment was subcloned into the EcoRI/HindIII cloning site of the pQE-60 plasmid (Qiagen). Escherichia coli strain Top10F' (Invitrogen) was used for transformation. Expression of the protein was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma). The protein was purified by affinity chromatography using a 5 ml HiTrap Heparin HP column (GE Healthcare).

Protein crystallization was performed in hanging-drop vapour-diffusion experiments by mixing 1 μl protein solution (1 mM rat FGF1 solution containing 0.5–1 mM SOS) and 1 μl reservoir solution (10% PEG 6000, 0.1 M citric acid pH 5.0). Crystals were obtained at room temperature within 2 d. X-ray data were collected at 100 K

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**Table 1**

Data-collection and processing statistics for the rat FGF1–SOS–FGF1 complex.

<table>
<thead>
<tr>
<th>Source</th>
<th>BW7A, EMBL, Hamburg</th>
</tr>
</thead>
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<tr>
<td>Wavelength (Å)</td>
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<tr>
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<tr>
<td>a (Å)</td>
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<tr>
<td>b (Å)</td>
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<tr>
<td>c (Å)</td>
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<tr>
<td>β (°)</td>
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<tr>
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<tr>
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<td>20.6</td>
</tr>
<tr>
<td>Rfree§ (%)</td>
<td>27.7</td>
</tr>
</tbody>
</table>

† Rmerge = \sum_{hkl} |I_o(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} I_o(hkl), where \langle I(hkl)\rangle is the mean intensity of an individual measurement of the reflection with Miller indices hkl and I_o(hkl) is the observed intensity of the reflection. ‡ Rwork = \sum_{hkl} |f_o(hkl) - f_c(hkl)| / \sum_{hkl} f_o(hkl), while f_o(hkl) and f_c(hkl) are the observed and calculated structure-factor amplitudes, respectively. § Rfree is equivalent to Rwork, but calculated with reflections omitted from the refinement process (5% of reflections were omitted).

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**Figure 1**

The rat FGF1–SOS–FGF1 ternary complex observed in the crystal structure. Both direct and SOS-mediated FGF1–FGF1 interactions are observed. A 2F_o – F_c electron-density map around SOS contoured at 1σ is shown in grey.
from a cryoprotected crystal (reservoir solution containing 20% glycerol) using synchrotron radiation (BW7A, EMBL, Hamburg). The data were indexed, integrated and scaled using the programs MOSFLM and SCALA (Collaborative Computational Project, Number 4, 1994). Crystal data and data-collection statistics are listed in Table 1.

The FGF1–SOS structure was determined by the molecular-replacement method using the program Phaser (Storoni et al., 2004). A crystal structure of rat FGF1 (molecule A, PDB code 2j3p; Kulahin et al., 2007) was used as a 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 95% of the residues. The missing residues were inserted manually using the program Coot (Emsley & Cowtan, 2004). Structure refinement was performed with the program REFMACS5 (Murshudov et al., 1999). Water molecules and a single SOS molecule 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).

Gel-filtration experiments were performed either in phosphate-buffered saline (PBS) or in PBS with 0.0362 mM SOS using a Superdex 75 column (GE Healthcare).

3. Results

3.1. Structure of the rat FGF1–SOS–FGF1 complex

The rat FGF1–SOS crystals contain two FGF1 molecules and one SOS molecule in the asymmetric unit (Fig. 1). Comparison of the present FGF1–SOS structure with that of rat FGF1 alone (Kulahin et al., 2007) revealed that FGF1 adopts the same conformation in both structures, with an overall r.m.s.d. of 0.83 Å on 129 Cα atoms.

SOS interacts with both FGF1 molecules in the asymmetric unit. The interaction area comprises both protein–protein and protein–SOS–protein contacts. The interactions in the crystal were analyzed.

![Image of protein structure](image-url)

Figure 2

(a) Ligplot showing interactions in the FGF1–SOS–FGF1 ternary complex. The interactions are dominated by salt bridges. (b) Stereo plot of a superposition of rat FGF1–SOS–FGF1 (blue C atoms) and bovine FGF1–SOS (green C atoms). Only residues within 5 Å from SOS are included and interacting basic residues are labelled.

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using the PISA server (Krissinel & Henrick, 2007). The FGFI(A)–SOS–FGF1(B) complex observed in the crystal is classified as a possible solution multimer, with a total buried accessible surface area of 1500 Å². Two additional minor FGFI–FGF1 crystal contact areas of 1180 and 960 Å² were found.

Direct hydrogen-bonding interactions between the two FGFI molecules and the highly charged SOS molecule are shown schematically in Fig. 2(a), which was generated with the programs LIGPLOT (Wallace et al., 1995) and HBPLUS (McDonald & Thornton, 1994). A total of 11 residues make interactions with seven of the eight sulfate groups of SOS. Seven of the 11 interacting residues are basic: three and four from the A and B chains, respectively. This suggests that SOS invokes strong electrostatic interactions within the ternary FGFI–SOS–FGF1 complex.

3.2. Gel filtration of rat FGFI in the presence of SOS

Gel filtration of rat FGFI produced a single symmetrical peak corresponding to the monomeric form of the protein (Fig. 3; red colour). When the gel-filtration column was pre-equilibrated with SOS-containing buffer (2:1 FGFI:SOS molar ratio), FGFI was eluted as a single peak with the elution volume reduced by 7 ml, corresponding to the dimeric form of the protein (Fig. 3; blue colour).

4. Discussion

The present study was undertaken to investigate if and how rat FGFI interacts with the heparin mimic SOS. From gel-filtration experiments, we estimated the molar weight of rat FGFI to be ~13 kDa (Fig. 3), compared with the calculated value of 17.4 kDa. After the addition of SOS (MW ≈ 1.1 kDa) to a 2:1 FGFI:SOS molar ratio, a single peak corresponding to a molar weight of 22 kDa was observed. Even though rat FGFI runs more slowly than expected, the experiment clearly suggests that a ternary FGFI–SOS–FGF1 complex is formed in solution.

The existence of the FGFI(A)–SOS–FGF1(B) ternary complex was confirmed by X-ray crystallography. A similar ternary complex was not observed in the bovine FGFI–SOS structure (Zhu et al., 1999). Superimposition of the rat FGFI–SOS–FGF1 complex and the bovine FGFI–SOS complex reveals that the binding modes are quite different (Fig. 2b) and that only two of the sulfates occupy similar positions. In the rat structure, the SOS furanose ring approximately occupies the position of the pyranose ring of the bovine complex. The residues forming the major sulfate-binding sites in bovine FGFI are Arg116, Lys118 and Arg122. Interestingly, whereas arginine is the consensus residue at this position in mammalian FGF molecules, it is a serine in rat FGFI. Instead, the major contributors to the sulfate-binding sites in rat FGFI are Lys113, Lys118, Arg122 and Lys128. This difference might explain why SOS-mediated FGFI dimerization is seen in rat and suggests that SOS functions differently in rats and cattle. A further consequence of SOS-mediated FGFI dimerization in rat is that it suggests that SOS-induced activation of rat FGFR follows the asymmetric model.

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References


