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The structural biology of growth factor receptor activation☆

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Abstract

Stimulation of cells by growth factors triggers cascades of signalling that result in cellular responses such as growth, differentiation, migration and survival. Many growth factors signal through receptor tyrosine kinases, leading to dimerization, *trans*-phosphorylation and activation of tyrosine kinases that phosphorylate components further downstream of the signal transduction cascade. Using insulin-like growth factor, nerve growth factor, hepatocyte growth factor and fibroblast growth factor as examples, we show that the globular architecture of the growth factors is essential for receptor binding. We describe how nerve growth factor (NGF) is a symmetrical dimer that binds four storage proteins (two α-NGF and two γ-NGF) to give a symmetrical hetero-hexameric 7SNGF organised around the β-NGF dimer. It binds the extracellular domains of two receptor molecules in a similar way, so dimerising the receptor. Hepatocyte growth factor/scatter factor (HGF/SF) probably binds its receptor as a dimer stabilised by interactions with heparan sulfate, and fibroblast growth factor (FGF) binds its receptor as a dimer cross-linked by heparan sulfate. Surprisingly, insulin and insulin-like growth factor (IGF) bind in the monomeric form to receptors that are already covalent dimers. We propose that, in general, weak binary interactions between growth factor and individual domains of receptors are enhanced by cooperative interactions with further receptor domains, and sometimes other components like heparan, to give rise to specific multi-protein/domain complexes.

Keywords: Growth factor; Receptor; Structural biology; Multiprotein complexes

1. Introduction

Stimulation of cells by growth factors can trigger complicated signalling cascades that result in cellular responses such as growth, differentiation, migration and survival. Many growth factors signal through receptor tyrosine kinases, by interacting with their extracellular domains. This is thought to lead to receptor dimerization and *trans*-phosphorylation, so activating tyrosine kinases that phosphorylate components further downstream of the signal transduction cascade [1]. Understanding the interactions of growth factors with their receptors and the nature of the activation process must ultimately depend on definition of the structures of complexes of the growth factors with their receptors and other obligatory components of the signalling process. Only then can we describe the

☆ This paper is dedicated to Dr John T. Edsall, in recognition of the inspiration and encouragement that he gave to researchers in protein structure and function.

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determinants of intermolecular interactions that give rise to specificity and begin to design molecules that will compete with the growth factors and be useful for therapeutic purposes.

Over the past 30 years, X-ray crystallography has defined the three-dimensional structures of growth factors and their complexes with receptors. These analyses have shown that the growth factors are usually small globular proteins that interact with multiple domains of the extracellular regions of the receptors. Receptor binding studies have shown that growth factors may bind, dimerise and activate their receptors either as monomers or dimers, and that dimerization of the growth factors can be mediated by co-receptors such as heparan sulfate [1].

Here, we focus on structural studies of growth factors and their receptors carried out in our laboratory. We show that in all cases the three-dimensional architecture of the growth factors is essential for receptor binding. We describe how nerve growth factor (NGF) is a symmetrical dimer that binds four storage proteins (two α-NGF and two γ-NGF) to give a symmetrical hetero-hexameric 7SNGF organised around the β-NGF dimer. It binds the extracellular domains of two receptor molecules in a similar way, so dimerising the receptor. Hepatocyte growth factor/scatter factor (HGF/SF) probably binds its receptor as a dimer stabilised by interactions with heparan sulfate, and fibroblast growth factor (FGF) binds its receptor as a dimer cross-linked by heparan sulfate. Surprisingly, insulin and insulin-like growth factor (IGF) bind in the monomeric form to receptors that are already covalent dimers. We propose that, in general, weak binary interactions between growth factor and individual domains of receptors are enhanced by cooperative interactions with further receptor domains, and sometimes other components like heparin, to give rise to specific multi-protein/domain complexes.

2. Results and discussion

2.1. Insulin, IGF and their receptors

The determination of the crystal structure of insulin [2] provided the first insights into the architecture of a polypeptide hormone/growth factor. Correlation of the structure with the results available on sequences, chemical modification, receptor binding and biological activity [3] indicated that the general topology of the insulin molecule was required for activity, and that a large region of the surface of the monomer was interacting with the receptor [4].

The insulin receptor and the homologous type 1 IGF receptor [5,6] are Mr 350 000 glycoproteins. They are composed of two disulfide-linked subunits, each containing a single transmembrane helix and a C-terminal, intracellular tyrosine kinase region. Although the structures of the catalytic tyrosine kinase domain of the insulin receptor in active and inactive states have been defined at high resolution by X-ray analysis [7], the structure of the extracellular region in complex with its ligand has proved more of a challenge. Comparative sequence analysis and modelling [8–10] and X-ray analysis [11] shows that the extracellular region of the receptors comprises two homologous globular domains (L1 and L2) flanking a cystine-rich domain, followed by three fibronectin III repeats. The L1 and L2 domains of IGFR have β-helical structures and are connected to the cystine-rich domain by flexible linkers, indicating that the relative positions of these domains in the crystal structure may not be retained in complex with the ligand. Studies with chimaeric insulin/IGF-I receptors [12] and alanine mutagenesis [13,14] suggest that the C-terminus of cystine-rich domain, the LI domain and a C-terminal peptide (residues 692–702) form the ligand-binding site.

2.2. NGF receptor and the hetero-hexameric 7S NGF storage complex

Nerve growth factor (NGF) is a neurotrophic factor that promotes the differentiation and survival of certain populations of neurons in the central and peripheral nervous systems. It is the prototype for a family of such factors, each with its own subset of target neurons. An increasing range of non-neuronal tissues have been found to be sensitive to NGF-induced responses, most notably immune-related haematopoietic cells [15].

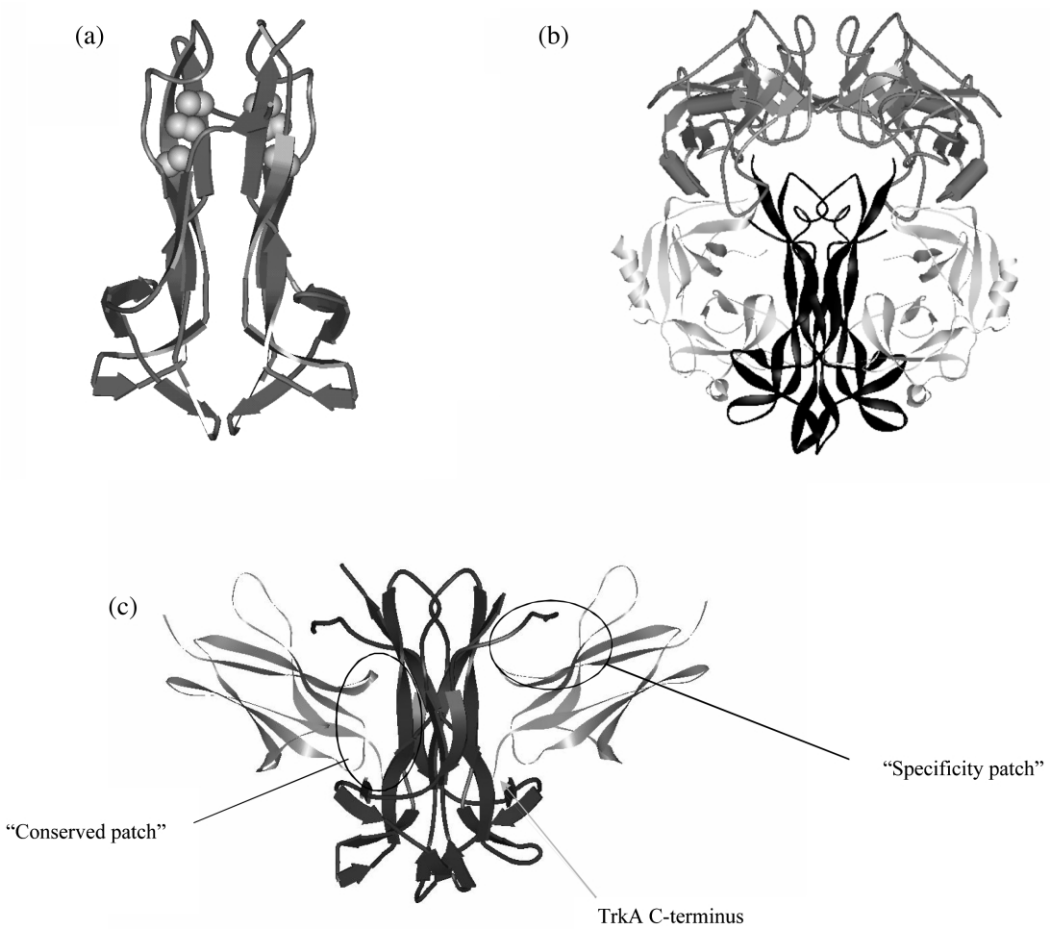

Fig. 1. Nerve growth factor: a comparison of receptor and binding protein interactions. (a) β-NGF dimer [17]. One dimer is shown, with the sulfur atoms from cysteine side chains making disulfide bridges shown as full spheres. (b) The 7S-NGF storage complex [18]. The β-NGF dimer is shown as ribbons in black, α-NGF as ribbons in light grey, and γ-NGF dimer schematically in dark grey. (c) β-NGF binding to the TrkA receptor membrane proximal domain [19]. NGF is shown as schematic in black, TrkA as ribbons in grey. The two main interaction regions are shown highlighted. One region is shown on each side for clarity: each TrkA monomer has both regions. The C-terminus of TrkA is highlighted to show the relationship to the membrane.

The active neurotrophin, β-NGF is biologically active as a dimer [16]. The 2.3 Å crystal structure of this dimer [17] (Fig. 1a) revealed that β-NGF adopts the cystine-knot fold, which was later shown to be common to transforming growth factor-β, platelet derived growth factor and several other growth factors. This fold is stabilised by three conserved intramolecular disulfide bridges. The dimer interface is principally hydrophobic, and buries 3032 $Å^2$ of surface area per dimer. This large buried surface area leads to the formation of the extremely stable dimer ($K_d = 10^{-13}$ M).

7S NGF is an $\alpha_2\beta_2\gamma_2$ complex in which the β-NGF dimer is associated with four serine proteinases of the glandular kallikrein family (two α-NGF and two γ-NGF subunits). γ-NGF is an active serine proteinase capable of processing the precursor form of β-NGF while α-NGF is an inactive serine proteinase, locked in the zymogen conformation. The 3.1 Å crystal structure of 7S NGF

[18] (Fig. 1b) shows that the dimeric γ-NGF (3033 Å^2 surface buried) bind close to the termini of the β-NGF dimer and make extensive interactions (2440 Å^2) with the β-NGF subunits. The two α-NGF subunits are bound on opposite sides of the β-NGF dimer, and they interact with the dimer via a short region of antiparallel β-sheet (2130 Å^2 surface buried in total). Two zinc ions, which stabilize the complex, are bound at the relatively small interfaces between the α-NGF and γ-NGF subunits. The regions of the β-NGF dimer that contact the α-NGF subunits overlap with those known to bind p75 neurotrophin receptor. The structure of 7SNGF shows how the two-fold axis of the central β-NGF dimer organizes the symmetry of this multiprotein growth factor complex.

NGF and its homologues are known to have two protein receptors on the surface of target cells. The high affinity receptors are a family of receptor tyrosine kinases, each specific for one NGF family member. Wiesmann et al. [19] crystallised NGF in complex with the membrane proximal extracellular domain of the NGF-specific receptor, TrkA. The structure (Fig. 1c) demonstrates how the TrkA can be dimerised by NGF dimers, and how highly homologous families of ligands and receptors (NGFs share ~50% identity to each other) can form highly specific interactions. Each TrkA monomer forms an immunoglobulin fold, and binds to one side of the β-NGF dimer, contacting analogous residues from the dimer. This brings the C-termini, which are closely linked to the transmembrane domain, into close proximity (Fig. 1c). There are two main sites of interface between TrkA and β-NGF. The main interface (burying ~750 Å^2 of TrkA surface) is to β-strand pairs exposed by NGF. This region of NGF is highly similar to that in the homologues. The second interface (with the N-terminal end of β-NGF) buries ~300 Å^2 of TrkA. The regions interacting here are highly varied in the family members, with only 20% identity for NGF and 25% for TrkA, and so are hypothesised to form a 'specificity patch' [20].

The structures of the β-NGF dimer, the 7S-NGF complex and the TrkA receptor domain complex show that a stable symmetrical dimer is very efficient organiser of the symmetry of multiprotein complexes in which it is involved. This brings together receptor dimers in a way that would facilitate transphosphorylation of receptor tyrosyl kinases and activation of the downstream signalling pathway. The structures also provide us with insights into how specific signalling can arise in multiprotein complexes, allowing reliable interactions in crucial developmental pathways and immune cell development.

2.3. Hepatocyte growth factor/scatter factor, HGF/SF, and its met receptor

Hepatocyte growth factor/scatter factor, HGF/SF, is essential for the development of the liver and other tissues in the mammalian embryo. The domain organisation resembles that of plasminogen, including an N-terminal domain (N), 4 kringle domains (K) and a catalytically inactive serine proteinase domain. [21–23]

A crystal structure at 2.3 Å resolution of the NK1 fragment, a naturally occurring partial agonist, has been defined [24]. NK1 is a splice variant of the polypeptide growth factor HGF/SF that consists of the N-terminal and first kringle domain. NK1 acts as a partial agonist and requires heparan sulfate or soluble heparin for full activity. In the crystals, the two subunits in the dimer interact through an extensive interface (2010 Å^2), although the fragment is a monomer in solution (Fig. 2a). A surface region, defined by mutagenesis experiments, that appears to interact with the receptor has been defined [24].

The crystal structures of NK1-heparin complexes show that heparin does not bridge sub-units (Fig. 2b), but appears to act electrostatically [25]. The heparin-binding site is located in the *N*-domain (Fig. 2c), where the major role is played by R73, with further contributions from main chain atoms of T61, K63 and G79 and the side chains of K60, R76, K62 and K58. Mutagenesis experiments show that heparin binding to this site is important for dimerization in solution and biological activity of NK1. Heparin also comes into contact with a patch of positively charged residues (K132, R134, K170 and R181) in the K domain. Mutation of these residues yields NK1 variants with increased bio-

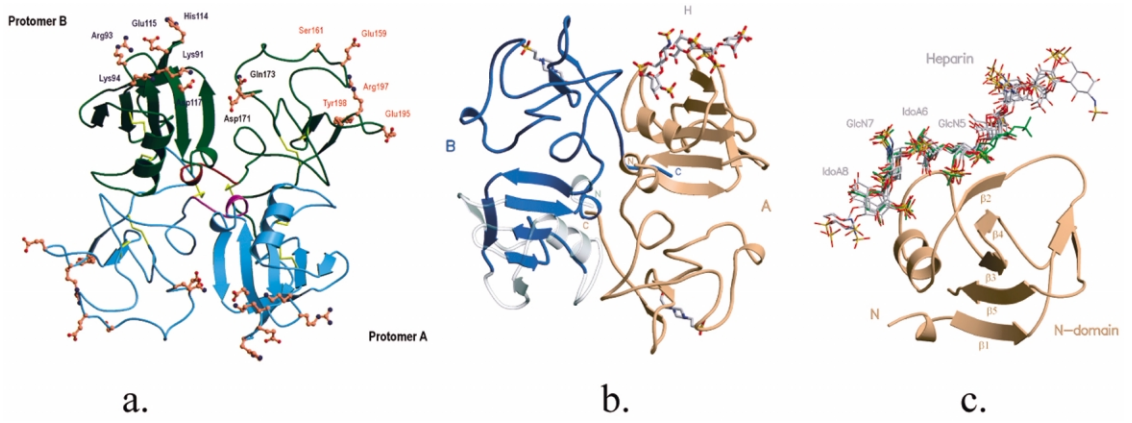

Fig. 2. The three-dimensional structure of HGF/SF NK1 fragment defined by X-ray analysis: (a) dimer of NK1 indicating a two-fold axis, with the residues implicated in receptor binding indicated on one protomer [24], (b) crystal structure of complex of one heparin fragments with NK1 dimer [25] and (c) the superposition of heparin fragments defined independently by X-ray analysis onto one *N*-domain of HGF/SF [25].

logical activity [25]. Thus, heparan sulfates play a complex role in biological activity of HGF/SF in which binding to the primary site in the N domain is essential for activity whereas binding to the K domain is inhibitory.

Thus, unlike NGF the HGF/SF NK1 molecules are monomeric in solution, but form dimers with heparin fragments. Interestingly, the same dimers are formed by NK1 on crystallisation in several different crystal forms ([24], unpublished observations). The dimers are presumably stabilised at the receptor by heparan sulfate or heparin, so leading to dimerisation of the receptor.

2.4. Fibroblast growth factor 1 (FGF1) in complex with its receptor (FGFR2) ectodomain and heparin.

Fibroblast growth factors (FGF) are a family of polypeptides with a pleiotropic range of physiological and pathological activities [26,27]. A considerable body of data has shown that the heparin-like proteoglycan polysaccharide heparan sulfate is required to allow activation of the receptor tyrosine kinase (FGFR) by FGFs [28,29]. Thus, it appears that association of FGF, FGFR, and heparan sulfate in a specific complex on the cell surface is essential for signal transduction. Although a wealth of information has been generated over the past decade by mutational studies, biophysical experiments, and cell growth assays, these data have not provided a reliable model for the FGF-FGFR-heparin complex (see, e.g. [26]). Previous structural studies have revealed binary complexes of FGF-heparin [30,31] and FGF-FGFR [32,33]. Whilst these structures help confirm the previous data from non-structural methods, they do not reveal three-dimensional information about the mode of ligand-induced FGFR oligomerisation in the presence of heparin. A further study [34] shows that hexasaccharides can bind to this 2:2 complex, but longer saccharides cannot be seen in this crystal form. We have defined the crystal structure of the FGFR2 ectodomain in a dimeric form, induced by simultaneous binding to FGF1 and a heparin decasaccharide [35]. The complex (Fig. 3) is assembled around a central heparin molecule linking two FGF1 ligands into a dimer that bridges between two receptor chains. Heparin binding is remarkably asymmetric and involves extensive contacts with both FGF1 molecules and one receptor chain. Protein–heparin interactions in the ternary complex lead to a large surface area buried, 2240 Å^2 (divided in 617 Å^2 with FGF1 and 631

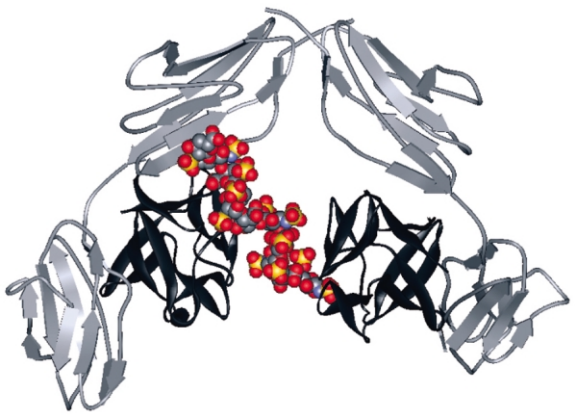

Fig. 3. FGF1, FGFR2 and heparin decamer in complex. FGF1 is displayed as a ribbon drawing in black, FGFR2 as schematic in grey, and heparin as all atom space-filling model. The C-terminus of FGFR2 (membrane proximal) is at the bottom of the picture. Note that heparin binds to one of the two FGFR2 molecules (ringed), whilst in the other protomer, the binding site is vacant (dashed box).

Å^2 with domain D2 in subunit A, and 992 Å^2 with FGF1 in subunit B). This comprises the close packing of several sulfate groups against the protein surface of ligand and receptor and the partial burial of lysine and arginine side chains upon binding to heparin. This, combined with another structural model and solution data, has led to the suggestion that FGFs prefer specific sulfation patterns of the heparan sulfate to which they will bind [35–37].

Thus, the structure of the FGF1–FGFR2–heparin ternary complex gives us insight into how a specific heparan sulfation pattern can be crucial to a protein-polysaccharide interaction [38]. It also shows that the essential role of heparin-like heparan sulfate in FGF signalling is based on the dimerisation of two FGFR:FGF 1:1 complexes.

3. Conclusions

Signalling systems, whether man-made or biological, need to achieve high specificity to be reversible and to achieve high signal to noise. In biological systems, this appears to be mediated by multiprotein systems. This strategy avoids the requirement for high-affinity binary complexes that would be difficult to reverse and which, in the overcrowded cell, would lead to excessive noise in the system. The robust development and survival of all multicellular organisms is a testament to the success of this process, and structural biology is uniquely placed to provide insight into how this is achieved.

Growth factor receptor interactions are key components of such signalling systems, responsible for communicating messages from outside the cell, across the membrane, and into the cytoplasm. The four growth factors, described here, serve as examples of the conditions that generally need to be met for efficient signalling.

First, interacting surfaces must be stable both as a component of, and apart from, the complex. Thus, they are rarely completely hydrophobic like the interfaces of permanent complexes that are retained during the lifetime of the protein. They contain a mixture of ion pairs, hydrogen bonds, water-mediated interactions and hydrophobic interactions. Thus, the FGFs, with mainly hydrophilic surfaces, are monomeric in solution, whereas β-NGF forms a permanent dimer, burying the hydrophobic surfaces, but producing a soluble ligand, again with a largely hydrophilic surface.

Secondly, most of the binary interactions are relatively weak. The instability of the FGF:FGFR 1:1 complex, compared to the greater stability of the FGF:FGFR:heparin 2:2:1 complex, is a good example of this. Another is the instability of the HGF NK1 dimer in the absence of heparin. This will decrease the chances of noise in the system, as the chances of fortuitous assembly of multiprotein complexes are much less. Nevertheless, cooperative interactions in the multiprotein complexes mean that high specificity is obtained, as shown by the hetero-pentameric FGF:FGFR:heparin.

During the signal transduction process, termination is as important as signal initiation, otherwise the sensitivity of the system would be lost. Failure to terminate can have severe consequences as demonstrated by the oncogenic mutants of signalling proteins, including several of these receptors. We, therefore, expect to see smaller buried surface areas in signalling systems, such as receptor-ligand

interactions, than in complexes that can be retained, such as the NGF dimer or Gβγ, or are permanently inhibitory such as Gβγ-phosducin [39]. Thus, the surface areas buried in signalling systems tends to be between 1500 and 2000 Å^2, whereas the surfaces involved in permanent complexes can be much larger (usually between 3000 and 5000 Å^2). These surfaces are often constructed from multiple domains or protomers that assemble cooperatively, as in the FGF:FGFR:heparin complex.

The assembly of the multiprotein complexes leads to the correct juxtaposition of two intracellular tyrosine kinase domains, allowing each subunit to phosphorylate the other and so activate signal transduction pathways. Each growth factor achieves this by a slightly different mechanism. In the case of insulin, the receptors are already covalently linked as a dimer. Insulin monomers appear to act to alter the conformation or relative orientations of the two kinase domains so that transphosphorylation can take place. On the other hand, the NGF receptor, TrkA, is monomeric in the absence of ligand. The stable NGF dimer is able to bring together two monomeric receptor chains to form the signalling complex. By analogy with the 7S-NGF complex and as shown in the structure of the complex of the NGF dimer with two fibronectin domains of the receptor, the two-fold symmetry of the NGF dimer is carried over into the multiprotein complexes. The HGF/SF NK1 fragment and FGFs use a different complexity to ensure faithful signalling. In both cases, an additional co-factor, heparan sulfate, is required to form the signalling complex. In the FGFR complex the heparin cross-links two FGF:FGFR complexes, surprisingly leading to approximate two-fold symmetry between the two FGF:FGFR complexes. In the case of HGF/SF, the effect of heparin is more subtle. Here it appears that the highly negatively charged molecule alters the local conditions such that the dimeric form of NK1 is favoured, without bridging between the dimer (as interactions with the site on the other molecule are inhibitory). Hence, the same co-factor, heparin, can have radically different effects on the different growth factors, and the different strategies again help to ensure specificity.

In conclusion, efficient signal transduction must maintain fidelity and decrease noise while amplifying the signal. The solution is to make signal transduction dependent on multiprotein complexes. If the binary interactions are weak, then chance collisions do not cause noise. On the other hand, multiprotein complexes can take advantage of cooperativity, so that the weak interactions in binary complexes are replaced by much stronger and more specific interactions in the higher complex.

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