Published in final edited form as:

Proteins. 2014 July ; 82(7): 1534–1541. doi:10.1002/prot.24523.

Structural Insights into FRS2 α PTB Domain Recognition by Neurotrophin Receptor TrkB

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Abstract

The fibroblast growth factor substrate 2 (FRS2) family proteins function as scaffolding adapters for receptor tyrosine kinases (RTKs). The FRS2 α proteins interact with RTKs through the phosphotyrosine-binding domain (PTB) and transfer signals from the activated receptors to downstream effector proteins. Here, we report the NMR structure of the FRS2 α PTB domain bound to phosphorylated TrkB. The structure reveals that the FRS2 α -PTB domain is comprised of two distinct but adjacent pockets for its mutually exclusive interaction with either non-phosphorylated juxtamembrane region of the FGFR, or tyrosine phosphorylated peptides TrkA and TrkB. The new structural insights suggest rational design of selective small molecules through targeting of the two conjunct pockets in the FRS2 α PTB domain.

Keywords

FRS2; PTB; SNT; FGFR; Trk; RTK; Neurotrophin Receptor; solution structure; NMR

INTRODUCTION

Signal transduction pathways through receptor tyrosine kinases (RTKs) play an important role in human physiology and pathophysiology including cancer and inflammation.¹ An increasing amount of evidence shows that scaffolding adapter proteins relay key molecular events in signal transduction initiating from cell-surface receptors to downstream elements.^{1,2,3} These scaffold adapter proteins involved in RTK signal transduction can be classified into two groups. The first group is comprised of docking proteins that have multiple tyrosine phosphorylation sites to anchor downstream signaling proteins. Members of this group include fibroblast growth factor (FGF) receptor substrate 2 (FRS2), a new SH2-containing sequence (Shc), Grb2-associated binder (Gab),^{4,5} insulin receptor substrate (IRS), and downstream of kinase (Dok)-family proteins.⁶ All of these scaffold adapter proteins act in the specification and/or amplification of the signal transduction pathway. The second group comprises of adapter proteins in a narrow sense. They have only SH3 or/and SH2 domains that bind signaling proteins such as Grb2, Crk and Nck.⁷

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The FGF receptor substrate (FRS) proteins represent a distinct family of docking/adaptor proteins.⁸ Members of this protein family include FRS2 α /SNT-1 and FRS2 β /FRS3/SNT-2 that share 49% sequence identity.⁷ FRS2 was identified as the first substrate of FGF receptor tyrosine kinase, and is also known as SNT-1 that stands for suc1-associated neurotrophic factor target one.^{9,10,11} Both FRS2 α and FRS2 β proteins consist of a myristoylation anchor and a PTB (phospho-tyrosine binding) domain in their N-terminus. Large regions at the C-terminus contain multiple tyrosine phosphorylation sites, which, upon phosphorylation, serve as binding sites for growth factor receptor binding protein-2 as well as for SH2 tyrosine phosphatase-2.¹² The PTB domains of the two FRS2 proteins share a high-degree sequence similarity with an overall 72% amino acid identity. The FGF family ligands and receptors are involved in a wide range of biological functions in development, tissue repair, angiogenesis and cellular homeostasis.^{13,14,15} Dysregulation of the FGF signaling axis via different mechanisms is a common feature of different tumor cell types,^{16,17} making the FGF pathway an attractive target for new therapy development.^{13,16} For example, expression of the PTB domain of FRS2 α inhibits anti-estrogen growth of breast carcinoma cells induced by over-expression of FGF1;¹⁸ Somatic rearrangements of the TrkA gene are found in papillary thyroid carcinomas and produce chimeric proteins. All of them contain a portion of TrkA, including tyrosine kinase domain. The resultant thyroid Trk oncoproteins trigger the expression of FRS2 α and FRS2 β in thyroid tumors.^{20,21}

FRS2 α proteins function as docking proteins downstream of different types of RTKs, including FGFRs, neurotrophin receptors (TrkA, TrkB, and TrkC),^{7,8} ephrin receptor (Eph), RET,^{22,23} and anaplastic lymphoma kinase (ALK).²⁴ FRS2 α proteins bind to RTKs through their PTB domain, which has been shown to be capable of interacting with unrelated sequences from different receptors in either a phosphorylation-independent or dependent manner. For example, the FRS2 α PTB domain binds to non-phosphorylated juxtamembrane region of the FGFR and FRS2 to the nucleophosmin-anaplastic lymphoma kinase (NPM-ALK).^{7,8,25} Interaction of FRS2 α PTB and the juxtamembrane region of FGFR does not include tyrosine residues. In contrast, binding of the PTB domain of FRS2 α to TrkA, TrkB, or RET is mediated by a tyrosine phosphorylated sequence that has an NPXpY motif (where X is any amino acid residue and pY is phosphorylated tyrosine) in the receptors and is dependent on activation of the RTKs.^{26,27} Interaction between FRS2 α and TrkA, TrkB or RET is dependent on phosphorylation of Y490, Y512 and Y1062 of the receptors, respectively. Although the affinities of the FRS2 α PTB domain binding to both FGFR1 and TrkA/B peptides are similar with K_d of 9.9 μ M, 5.2 μ M and 7.7 μ M for FGFR1, TrkB, and TrkA, respectively, the thermodynamics of the binding are very different.²⁵ For instance, FGFR1 recognition is governed by a favorable entropy contribution to the free energy of binding, whereas tyrosine-phosphorylated Trk binding is largely enthalpy-driven. The favorable entropy change of FGFR1 binding is due to structural reordering of the PTB domain, which results from an increase in protein conformational flexibility and/or changes in solvation at the complex interface when the complex formation buries more hydrophobic surface. On the other hand, the large favorable enthalpy-contribution associated with Trk binding is due to the intermolecular electrostatic, hydrogen-bonding, and hydrophobic contacts established in the complex.²⁵ In order to identify the molecular basis of the PTB domain of FRS2 α recognition of Trks and FGFR peptides, we solved the three-dimensional

solution structure of the FRS2 α PTB domain in complex with a TrkB peptide. We examined FRS2 α interactions with FGFR and TrkB peptides, and compared their structural and binding mechanisms with those of the other PTB domains, and analyzed similarities and differences between FRS2 α binding to different peptides.

MATERIALS AND METHODS

Sample Preparation

The human FRS2 α PTB domain (residues 8–122) was cloned, expressed and purified using a procedure as described previously.²⁷ cDNA fragments encoding FRS2 α PTB domain and TrkB (residues 493–515) were sub-cloned into a modified pET28b (Novagen) and pGEX 4T1 (GE Healthcare) vectors respectively. Additionally, three recombinant TrkB peptides, i.e. TrkB-short (residues 505–516), TrkB-medium (residues 493–518) and TrkB-long (residues 453–518), were prepared. These peptides were purified and phosphorylated *in vitro* using the insulin receptor's cytoplasmic kinase domain (residues 953–1355) (cDNA construct was a gift from Dr. Ora Rosen) using an established method.²⁸ His-Tagged FRS2 α PTB and GST-TrkB were over-expressed in *E. coli* pRIL plasmid BL21-CodonPlus cells and induced with 0.3 mM isopropyl- β -D-thiogalactopyranoside at 18°C. His-Tagged SNT1 PTB and GST TrkB were purified by HiTrap IMAC or GSTrap column (GE Healthcare). After removing His-Tag or GST with thrombin treatment, protein samples were further purified with Superdex 75 column (GE Healthcare). Uniformly ¹⁵N-, ¹⁵N/¹³C-labeled proteins were prepared by growing the bacteria in M9 minimal medium containing ¹⁵NH₄Cl with or without ¹³C-glucose. Uniformly ¹⁵N/¹³C-labeled proteins and fractionally deuterated proteins were prepared using M9 medium with 75% ²H₂O.

NMR spectroscopy

The FRS2 α PTB domain/TrkB peptide (493–515) complex was used for structure determination. All NMR spectra were acquired at 25°C on Bruker 500, 600, and 800 MHz spectrometers equipped with z-gradient triple-resonance cryoprobes. The backbone ¹H, ¹³C, and ¹⁵N resonances were assigned using standard three-dimensional triple-resonance HNCA, HN(CO)CA, HN(CA)CB, and HN(COCA)CB experiments.²⁹ The side-chain atoms were assigned from three-dimensional HCCH-TOCSY, HCCH-COSY, and (H)C(CO)NH-TOCSY data.²⁹ The NOE derived distance restraints were obtained from ¹⁵N- or ¹³C-edited three-dimensional NOESY spectra. The TrkB peptide was assigned from two-dimensional HTOCSY, HNOESY, HROESY, and ¹³C/¹⁵N-filtered HTOCSY. The intermolecular NOEs used in defining the structure of the complex were detected in ¹³C-edited (F1), ¹³C/¹⁵N-filtered (F3) three-dimensional NOESY spectra (unlabeled TrkB peptide bound to ¹³C/¹⁵N-labeled PTB protein; ¹³C/¹⁵N-labeled peptide to unlabeled protein).³⁰ Spectra were processed with NMRPipe and analyzed using NMRVIEW.^{31,32}

Structure calculations

Structures of the FRS2 α PTB domain/TrkB peptide were calculated with a distance-geometry simulated annealing protocol with CNS. Initial protein structure calculations were performed with manually assigned NOE-derived distance constraints. Hydrogen bond distance, ϕ and ψ dihedral-angle restraints were from the TALOS+ prediction,³³ and they

were added at later stage of structure calculations for residues with characteristic NOE patterns.³⁴ The converged structures were used for the iterative automated NOE assignment by ARIA refinement. Structure quality was assessed with CNS, ARIA and PROCHECK analysis.^{35,36,37} Total 192 intermolecular NOE-derived distance restraints were added in the structure determination of the PTB/TrkB peptide complex. A family of 200 structures was generated and 20 structures with the lowest energies were selected for the final analysis.

RESULTS AND DISCUSSION

Structure of the FRS2α PTB Domain with TrkB Peptide

Nuclear magnetic resonance (NMR) spectroscopy study was conducted using a 1:1 complex of the FRS2α PTB domain (residues 8–122) and a 23-residue tyrosine phosphorylated peptide (residues G493-I515, GPDAVIIGMTKIPVIENPQ-pY-FGI, where pY is phosphorylated tyrosine) derived from the FRS2α binding site (phosphotyrosine 512) of the nerve growth factor TrkB (TrkB-23mer) (Figure 1A, 1B). The well-defined three-dimensional solution structure of this complex was determined using a total of 2,544 NMR-derived distance and dihedral angle restraints (Figure 1A). Superimposition of an ensemble of the 20 final structures of the complex is depicted in Figure 1C, and the final RMSDs are 0.24Å and 0.57Å for secondary backbone and heavy atoms, respectively (Table I). The protein structure consists of seven β-strands and one α-helix. The seven β-strands are folded into two groups of anti-parallel β sheets (β1–β4 and β5–β7) and sandwiched together in a nearly orthogonal arrangement. The amphipathic α-helix end is capped at C-terminal end (Figure 1C, 1D). These structural features are typical for the canonical PTB domain fold.²⁵

The dissociation constant (K_d) of the protein/peptide (TrkB-13mer, PVIENPQ-pY-FGIT) complex was previously determined to be about 5 μM at pH=7 using the isothermal titration calorimetry (ITC) technique.²⁵ NMR ¹H-¹⁵N heteronuclear single quantum coherence (HSQC) spectroscopy titration showed that a TrkB peptide (TrkB-long) comprising residues A453-S518 interacts better with the FRS2α PTB domain than shorter TrkB peptides (TrkB-short, residues P505-T516, or TrkB-medium, residues G493-S518) (Figure S1). With the TrkB-long peptide, the ¹H-¹⁵N HSQC spectrum of the protein has the best spectral quality and strongest chemical shift perturbation (estimated K_d of <1 μM); the TrkB-medium peptide binding ¹H-¹⁵N HSQC is the second best (estimated K_d of ~2 μM) whereas the TrkB-short peptide is the worst among the three (Figure S1).

In the complex structures of the FRS2α PTB with the TrkB-23mer peptide, the peptide interacts extensively with the FRS2α PTB domain by adopting an unusual conformation. The N-terminal segment AVII of the peptide folds back and forms an anti-parallel β sheet (β1'–β2') with the middle segment TKIP of the same peptide. There are three hydrogen bonds between the backbone atoms of the β1'–β2' sheet, and large number of NOEs characteristic of anti-parallel β sheet are observed. Side chains of Ala496/Pro505, Val497/Ile504, Ile498/Lys503 and Ile499/Thr502 interact extensively with each other (Figure 2A). The anti-parallel β sheet of the peptide is orthogonally arranged on top of protein's β5–7 sheet to form a β-sandwich structure, whose interactions are primarily hydrophobic (Figure 2C, 2D). Side chains of Val497 (pY–15), Ile499 (pY–13), and Thr502 (pY–10), Ile504 (pY–8) and Val506 (pY–6) interact with otherwise solvent-exposed hydrophobic residues Leu71,

Ser73, Ile86, and Ala88 of the protein (Figure 2). Complementary electrostatic interactions are also observed between Thr502 and Asp68, Asp495 and Arg64 (Figure 2). These results suggest that both hydrophobic and electrostatic interactions are important for FRS2 α PTB and TrkB association.

The C-terminal PVIE (β 2'-strand) sequence of the peptide forms an anti-parallel β sheet with β 5 of the FRS2 α PTB domain and interacts with the groove formed by β 5 and α 1. Three inter-molecular hydrogen bonds bridging between backbone atoms of β 2' and β 5 and a large number of NOEs characteristic of the anti-parallel β -sheet are observed of the complex (Figure 2B). Side chains of Pro505, Val506 and Ile-507 interact extensively with Leu62, Tyr65, Tyr67, Asp68, Phe98 and Met105 in a hydrophobic core formed between β 5 and α 1. The conserved Pro505 is located in the middle of the β -strand of the peptide and forms a kink that maintains the appropriate hydrogen bonds in register between β -strands. The phosphotyrosine pTyr512 of C-terminal peptide forms electrostatic interaction with positively charged residues of Arg63, Arg78 and Cys61.

Comparison to the FRS2 α PTB / FGFR1 complex

The FRS2 α PTB domain is capable of binding not only to the canonical NPXpY motif in Trks, but also to a juxtamembrane region in FGFR containing no tyrosine or asparagine residues.^{25,27} However, the solution structure of the FRS2 α PTB domain/TrkB shows different binding mechanism and thermodynamics from the complex of FRS2 α PTB/the human FGFR1 peptide HSQMAVHKLAKSIPLRRQVTVS.²⁷ First, the solution structure of the FRS2 α PTB/FGFR1 peptide complex shows that the peptide wraps around the protein with an unusual backbone conformation containing two nearly 90° turns that are oriented orthogonal to each other (Figure S2C, S2D). The peptide embeds its N-terminal MAVH segment into a large hydrophobic cavity bounded by the three loops connecting β 1/ β 2, β 3/ β 4 and β 6/ β 7. But the structure of FRS2 α PTB/TrkB shows that the N-terminal AVII segment folds back to form an anti-parallel β -sheet (β 1'- β 2') with its own TKIP middle segment (Figure 2). This β -sheet binds to otherwise solvent-exposed hydrophobic residues on the protein surface of the second β -sheet, which corresponds to the LAKSIPL sequence located in the center of the FGFR1 peptide (Figure S2A, S2B). With deletion of the N-terminal AVII segment of TrkB peptide, ¹H-¹⁵N HSQC shows a reduction of the peptide binding to the protein (Figure S1).

Second, The C-terminal QVTVS segment of the FGFR1 peptide adopts an anti-parallel β -strand sandwiched between β 5 and β 8, forming an intermolecular β -sheet. This unique β 8, extended from the C-terminal α -helix (α 1), is not found in other PTB domains.²⁵ Side chains of Val427, Thr428 and Val429 of FGFR1 show hydrophobic interactions with Ile110, Val112 and Val113 of β 8 strand. Complementary electrostatic interactions are also observed between peptide and β 8: Arg425 interacts with Glu114 and Glu119, while Gln426 with Glu115. Mutation of Val429 completely eliminates peptide binding to the protein, while mutations of Arg425 and Val427 also significantly reduced the binding²⁷. The corresponding binding sequence in TrkB peptide is IPVIE, forming the extended part β 2'-strand that interacts in an anti-parallel way with β 5 strand of the protein. The Pro505 of TrkB corresponds to Val427 in FGFR1, it is located in the middle of the β 2'-strand and

forms a kink that breaks a turn to the peptide β 2'-strand. Therefore, the side chain of Ile504 is above β 5/ β 6 sheet and cannot form the anti-parallel alignment like Gln426 of FGFR1. Because of the anti-parallel β -sheet (β 1'- β 2') formation, the interaction β 1' strand β 2' partially blocks potential binding space between C-terminal protein with the β 2' strand, the protein's region corresponding to β 8 strand in FRS2 α PTB/FGFR1 complex has no interaction with the β 2'-strand of TrkB peptide and it is disordered. No intermolecular NOEs were observed between this region and the TrkB peptide. As a result, the deletion of this region has no effect to the binding of TrkB with FRS2 α PTB domain.²⁵

Third, the C-terminal of the TrkB phosphopeptide in the FRS2 α complex is important for the peptide binding. The phosphotyrosine pTyr512 interacts with positively charged residues Arg63, Arg78 and Cys61 in the protein (Figure S2A, S2B), Gln511 shows hydrophobic interaction to Leu60 and Met105 of the protein, Pro510 to Met105, and Asn509 to Leu62, Tyr65 and Met105 (Figure 2), but the C-terminal of FGF1 peptide beyond the QVTVS segment has no effect on the FRS2 α PTB domain (Figure S2C, S2D). Finally, the thermodynamics of the FRS2 α PTB domain binding to the two peptides are different: FGFR1 recognition is governed by a favorable entropic contribution to the free energy of binding, whereas tyrosine-phosphorylated TrkB binding is largely enthalpy-driven. The favorable entropy change in FGFR1 binding is due to structural reordering of the PTB domain, resulting from an increase in protein conformational flexibility and/or changes in solvation at the complex interface that accompanies the burial of more hydrophobic surface upon complex formation. The large favorable enthalpy contribution associated with TrkB binding is due to electrostatic, hydrogen bonding and inter-molecular hydrophobic contacts established in the complex from phosphotyrosine Tyr512 to positively charged residues Arg63 and Arg78.

Comparison to Shc and IRS-1 PTB domains

All three PTB domains of FRS2, IRS-1 and Shc recognize NPXpY-containing peptides in a thermodynamically similar manner, but only the FRS2 α PTB domain can interact a non-phosphopeptide, and the binding is thermodynamically different from that of NPXpY-containing peptide.^{25,38,39} The FRS2 α and IRS-1 PTB domains share high homology in overall sequence and structural topology. The Shc PTB domain shows very low sequence similarity and cannot reliably be aligned with those of FRS2 α and IRS-1 in the absence of structural information, but the overall fold of three PTB domains of IRS-1, Shc and FRS2 α PTB domain are similar (Figure S3A–S3C). However, there are some differences between the three proteins. Compared to the PTB domain of FRS2, the Shc PTB domain is larger and contains a long insert between β 1 and β 2, which includes a 5-turn α -helix and additional pair of β -strands (Figure S3B). The IRS-1 PTB domain has a one-turn helix between β 1 and β 2 (Figure S3C).

Three PTB domains of FRS2, IRS-1 and Shc display distinct binding specificities for NPXpY peptides, which can be explained by their structures. IRS-1 recognizes the (pY-6) and (pY-8) residues of the IL-4 receptor peptide through interactions with a hydrophobic site on the surface of the second β -sheet. The analogous site in Shc is not available for peptide binding, because it is covered by a loop and the N-terminal portion of α -helix (α 2). In the

FRS2 α PTB-TrkB complex, the N-terminal of TrkB peptide folds back to form an anti-parallel β -sheet, which engages more hydrophobic interactions with the second β -sheet of FRS2 α PTB domain. The protein binds to more peptide residues than IRS-1, it recognizes the peptide at Val506 (pY-6), Ile504 (pY-8) but also Thr502 (pY-10), Met501 (pY-11), Ile499 (pY-13) and Val497 (pY-15). Consistent with our ^1H - ^{15}N HSQC studies, addition of (pY-8), (pY-10), (pY-11) and (pY-13) residues to the TrkB peptide improve the binding to the FRS2 α PTB domain, which is similar to the result in adding (pY-6) and (pY-8) IL-4 peptide binding to IRS-1. In both FRS2 α and Shc, the long side chain of Ile (pY-5) of the Trk peptide binds in a deep hydrophobic pocket, which is located between β 5 and the C-terminal α -helix. The corresponding residue of IL-4 receptor peptide Ala (pY-5) is a small hydrophobic residue; it binds to the surface of IRS-1. In three PTB domains, two arginines are important for the binding to the phosphotyrosine, but they are located in different regions of the sequences. Arg175 in Shc (which is structurally homologous to Arg227 of IRS-1 and Arg78 of FRS2) is near β 7 rather than β 6. In fact, Arg227 of IRS-1 and Arg78 of FRS2 α are in a location more closely related to Lys169 in Shc. The other arginine of Shc involved in binding to the phosphotyrosine, Arg67, is located in the loop between β 1' and the second α -helix- a region that is missing in IRS-1 and FRS2. The corresponding arginines in IRS-1 (Arg212) and FRS2 α (Arg63) are located at the N-terminal of β 5. In FRS2 α PTB, the Cys61 on the loop between β 4 and β 5 shows additional electrostatic interaction to the phosphotyrosine of the TrkB peptide, while the Lys169 in Shc has the similar effect. Finally, in the IRS-1-peptide or FRS2-peptide complexes, the C-terminal end of the phosphopeptide extends away from the protein. In contrast, the C-terminal of the TrkB phosphopeptide in the Shc complex folds back on itself and interacts with a hydrophobic site in the protein, explaining Shc's preference for binding to aromatic residues at the (pY+1) position.

The FRS2 α PTB domain can bind specific peptides with or without tyrosine-phosphorylated residues. Compared to the complex structures of FRS2 α PTB with FGFR1 peptide, our new three-dimensional solution structure of FRS2 α PTB domain bound to the TrkB peptide reveals that the PTB domain relies upon different interaction surfaces when it selectively and specifically binds to the TrkB. The PTB domains of FRS2 α proteins bind to unphosphorylated peptides at the juxtamembrane domain of the FGF receptor; the binding is stable with or without activation of the receptors. FGFR1 recognition is governed by hydrophobic and aromatic interactions of the peptide to second β -sheet. The C-terminal QVTVS sequence of peptide adopts an anti-parallel β strand; the unique β 8-strand, extended from usually unstructured NVVEE segment from the C-terminal α 1-helix is not found in other known PTB domain structures. The narrow and deep cleft formed by β 8, β 5 and α 1 of the PTB domain engages seven hydrogen bonds to the backbone β '-strand of the peptide and is able to clip the peptide tightly and selectively (Figure S2B). However, the positively charged residues of Arg63 and Arg78 in the PTB domain are intact and could still engage interactions with an available phosphorylated tyrosine. On the other hand, binding between the PTB domain and neurotrophin receptor TrkA and TrkB, or RET is mediated by tyrosine phosphorylated peptides and is dependent on activation of the RTKs. The FRS2 α PTB protein and TrkB binding is established by electrostatic contacts, hydrogen bonding as well as intermolecular hydrophobic associations. The phosphorylated tyrosine 512 of TrkB interacts with positively charged and solvent-exposed residues Arg63 and Arg78, and this

recognition is the key for the binding affinity and selectivity between the peptide and the protein (Figure S2A, S2B). The N-terminal of TrkB peptide forms its own anti-parallel β -sheet ($\beta 1'$ – $\beta 2'$), which interacts hydrophobically to the second β -sheet of the protein. The middle PVIE segment TrkB peptide forms an anti-parallel β -sheet with $\beta 5$ of the protein and interacts with the groove formed by $\beta 5$ and $\alpha 1$, but there is no $\beta 8$ from C-terminal to bind to the peptide like FGFR1 peptide binding.

The structural analysis of the FRS2 α PTB domains in complex with FGFR1 or TrkB peptide reveals that the FRS2 α PTB domain has two related but independent sub-pockets around the peptide binding site; one is hydrophobic, formed by $\beta 8$, $\beta 5$ and $\alpha 1$ for FGFR1 recognition. This hydrophobic pocket is entropy driven resulting from protein structural reordering and restricted dynamic mobility of the peptide in the complex. The other pocket is polar, formed by electrostatic and hydrogen-bond interaction of TrkB pTyr-512 to Arg-63 and Arg-78 of the protein; the binding is enthalpy driven. To take the benefit of both the hydrophobic and electrostatic pockets (Figure S2E, S2F), a decoy peptide may be constructed with elements from the FGFR1 and TrkB peptides. For instance, one could add the NPXpY motif from TrkB to the C-terminal portion of the FGFR1 peptide, yielding a peptide of HSQMAVHKLAK-SIPLRRQVTVS-NPQpY. Such a new peptide may retain the association properties from both FGFR1 and TrkB peptides to the FRS2 α PTB domain, result in high affinity and selectivity. Such a new peptide may be used as a probe in a high throughput screens of compounds toward the development of inhibitors that block the FRS2 α PTB domain activity in receptor-mediated cell signaling required for cancer progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to acknowledge the use of the NMR facility at the New York Structural Biology Center. We thank Drs. Nicolas Babault, Mark Vargas, Qiang Zhang and Guangtao Zhang for helpful suggestions and critical reading of the manuscript, and thank Dr. Jamel Meslamani for computer technical support. Resonance assignments and 3D NMR NOESY peak lists for corresponding NMR structures are being deposited at the BioMagResBank (19562), the structural coordinates have been deposited in the Protein Data Bank (2MFQ). This work was supported in part by the research grants from the National Institutes of Health (to M.-M.Z.).

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Figure 1. Structure of the FRS2 α PTB domain with TrkB complex

A. Ribbon depiction of the lowest energy NMR structure of the FRS2 α PTB domain/TrkB peptide complex.

B. Ribbon diagram of the of the FRS2 α PTB domain/TrkB complex from the top view of the protein, which is rotated ~ 90 degree from the orientation of (A).

C. The backbone atom superposition of the final 20 NMR-derived structures of the complex. The figure shows the FRS2 α PTB domain residues 11–106 and the TrkB peptide residues 493–516. The terminal residues, which are structurally disordered, are omitted for clarity. The orientation of the structures is the same as (A).

D. Electrostatic potential surface representation of the FRS2 α PTB domain bound to the TrkB peptide (Green). The orientation is the same as (A). The electrostatic potential calculation was performed in PyMol (v1.2) using APBS modules.

Figure 2. The TrkB peptide forms anti-parallel β sheet (residues 496–505) and molecular interface between the protein and the peptide

A. Ribbons diagram of anti-parallel β sheet of the TrkB peptide interacting with the protein $\beta 2$ sheet surface. The peptide and protein are labeled in green and grey.

B. Expanded diagram of key side-chain interaction from the anti-parallel β sheet TrkB peptide. The peptide orientation in the stick representation is depicted as in the ribbon diagram on (A). The peptide elements are colored in blue, green, red and yellow. The hydrogen bonds are indicated with dotted lines.

C. The stick diagram showing side-chain and backbone interactions in the FRS2 α PTB domain/TrkB peptide complex. Respectively. PTB domain residues involved in peptide binding are labeled and colored yellow.

D. Surface-filling diagram of the FRS2 α PTB domain bound to the TrkB peptide (shown in stick models), respectively. The peptide residues are colored in green; PTB domain residues involved in peptide binding are labeled and colored red.

Table I

Summary of Restraints and Statistics of the Final 20 NMR Structures of the FRS2 α PTB Domain/TrkB Complex

Distance constraints	
Total NOE	2,544
Intra-residue	1,006
Inter-residue	1,538
Sequential ($ i - j = 1$)	384
Medium-range ($1 < i - j \leq 5$)	283
Long-range ($ i - j > 5$)	871
Inter-molecular constraints	192
Hydrogen bonds	50
Total dihedral angle restraints	
Phi angle	93
Psi angle	93
Ramachandran Map Analysis (%) [*]	
Most favored regions	82.8
Additional allowed regions	17.2
Generally allowed regions	0.0
Disallowed regions	0.0
Structure statistics	
Violations (mean $\pm$ s.d.)	
Distance constraints (Å)	0.073 ± 0.0033
Dihedral angle constraints (°)	1.19 ± 0.1
Max. dihedral angle violation (°)	1.37
Max. distance constraint violation (Å)	0.08
Deviations from idealized geometry	
Bond lengths (Å)	0.0079 ± 0.00012
Bond angles (°)	0.91 ± 0.017
Impropers (°)	2.2 ± 0.12
Average pairwise r.m.s. Deviation (Å) ^{**}	
Heavy	0.57 ± 0.059
Backbone	0.24 ± 0.055

^{*} Procheck analysis was done for protein residues 13–20, 26–32, 37–40, 47–52, 56–61, 64–70, 78–85, 87–101, 496–498 and 502–510.

^{**} The residues used in protein/peptide complex's RMSD calculations are 11–70, 78–103 and 495–512.

Pairwise r.m.s. deviations were calculated among 20 refined structures of total 200 structures.