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Identification of Brain-Specific Angiogenesis Inhibitor 2 as an Interaction Partner of Glutaminase Interacting Protein

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Abstract

The vast majority of physiological processes in living cells are mediated by protein-protein interactions often specified by particular protein sequence motifs. PDZ domains, composed of 80–100 amino acid residues, are an important class of interaction motif. Among the PDZ-containing proteins, Glutaminase Interacting Protein (GIP), also known as Tax Interacting Protein TIP-1, is unique in being composed almost exclusively of a single PDZ domain. GIP has important roles in cellular signalling, protein scaffolding and modulation of tumor growth and interact with a number of physiological partner proteins, including Glutaminase L, β -Catenin, FAS, HTLV Tax, HPV E6, Rhotekin and Kir 2.3. To identify the network of proteins that interact with GIP, a human fetal brain cDNA library was screened using a yeast two-hybrid assay with GIP as bait. We identified Brain-specific Angiogenesis Inhibitor 2 (BAI2), a member of the adhesion-G protein-coupled receptors (GPCRs), as a new partner of GIP. BAI2 is expressed primarily in neurons, further expanding GIP cellular functions. The interaction between GIP and the carboxy-terminus of BAI2 was characterized using fluorescence, Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopy assays. These biophysical analyses support the interaction identified in the yeast two-hybrid assay. This is the first study reporting BAI2 as an interaction partner of GIP.

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Author contributions:

S.Z. and M.O.; Helped design and performed experiments, analysed data, M. D; interpretation of data., M. B; BLAST search to find the peptide sequence., Z. T. and S. M; designed the project, analysed data and prepared the manuscript.

Dedication:

The authors dedicate this article in the loving memory of Dr. Marie W. Wooten, Dean and Professor of the College of Sciences and Mathematics (COSAM) at Auburn University, who tragically passed away on November 5, 2010. Dean Wooten was our mentor, colleague, friend, and role model. She will be deeply and painfully missed, yet celebrated, honored, and never forgotten.

Keywords

Glutaminase Interacting Protein; Yeast-two-hybrid; Brain-Specific Angiogenesis; Inhibitor 2

1. Introduction

Glutaminase interacting protein (GIP) [1], also known as tax interacting protein-1 (TIP-1) [2], is a 13.7 kDa PDZ domain-containing protein. PDZ domains are one of the most important protein-protein interaction modules in nature [3]. PDZ domain-mediated interactions contribute to cell signalling, adhesion and receptor and ion transporter function [4]. PDZ domains often act as scaffolds, specifying protein interactions required for the formation of multimeric complexes [5]. The diversity of PDZ domain-protein interactions and their involvement in maintenance of normal physiological functions of the body, are significant in the context of clinical disorders. Several human diseases are known to occur as a result of inappropriate protein-protein interactions, which in turn affect gene expression and regulation, transport of biomolecules across the membranes, cell adhesion, antigen recognition and signal transduction [6].

The binding pocket of PDZ domains and the mode of binding to the interacting partner proteins are each well characterized [4,7,8,9]. The GLGF motif present in the binding pocket of PDZ domains plays a major role in binding interactions with the target protein. PDZ domains were therefore previously referred to as GLGF repeat domains. PDZ domains exhibit sequence specificity towards the unstructured C-terminal ends of their interacting protein partners. Peptides representing these C-terminal recognition motifs have been shown to act as surrogates for their corresponding partner proteins *in vitro*. Several classes of PDZ domains have been reported based on this specificity [4,7–9].

GIP is an unusual class I PDZ domain protein in the sense that it is solely composed of a single PDZ domain [5]. Structurally, GIP is made up of two α -helices and eight β -strands [9]. GIP is also striking for the promiscuity of its binding profile. Some of the reported interacting proteins include Glutaminase L, β -Catenin, FAS, HTLV Tax and HPV E6, which are involved in signalling pathways, energy generation pathways or oncogenic processes [1,2,7–9].

GIP is known to function as a key scaffolding protein in the mammalian brain [10], contributing to the bioenergetics of both normal and cancer cells through its interaction with Glutaminase L [1]. GIP may also mediate normal brain cellular functions through interactions with other as yet unidentified partner proteins. To fully understand the mechanism of function of GIP in the brain, it is necessary to identify the proteins that interact with GIP in brain cells.

Among the various methods for the investigation of novel protein-protein interactions, the yeast two-hybrid (Y2H) method, developed by Song and Fields in *Saccharomyces cerevisiae* (baker's yeast), is a powerful approach with several advantages over traditional biochemical approaches [6, 11]. It involves the expression of two proteins being assessed for interaction within the yeast cell nucleus. In this study we used the yeast two-hybrid system to screen a human fetal brain cDNA library for GIP-interacting proteins. We identified Brain-specific angiogenesis inhibitor 2 (BAI2) as a novel interacting partner of GIP. CD, fluorescence and NMR characterization of the interaction between GIP and a peptide representing the BAI2 C-terminus as surrogate support the yeast two-hybrid assay results identifying BAI2 as a protein recognized by GIP.

2. Materials and Methods

2.1. Yeast strains and media

Two-hybrid reporter yeast strains used in this study were AH109 (*MATa*, *trp1-901*, *leu2-3*, *112*, *ura3-52*, *his3-200*, *gal4Δ*, *gal80Δ*, *LYS2::GAL1_{UAS} GAL1_{TATA}-HIS3*, *GAL2_{UAS}-GAL2_{TATA}-ADE2*, *URA3::MEL1_{UAS}-MEL1_{TATA}-lacZ*, *MEL1*) and Y187 (*MATa*, *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3*, *112*, *gal4Δ*, *met*, *gal80Δ*, *MEL1*, *URA3::GAL1_{UAS}-GAL1_{TATA}-lacZ*) (Clontech Mountain View, CA). Yeast cells were cultured at 30°C in YEPD + adenine or SD medium [12]. All media reagents were obtained from DIFCO Laboratories or Sigma Chemical Co.

2.2. Two-hybrid Screen

A *Bam*HI/*Sal*I fragment encoding the GIP open reading frame (ORF) was removed from the recombinant pET-3c/GIP plasmid [13] and inserted into the yeast pGBKT7-GAL4 DNA Binding Domain (BD) vector (Clontech, Mountain View, CA) to generate the plasmid pGBKT7-GIP. Yeast strain Y187, pre-transformed with a human fetal brain cDNA library in the Gal4 activation domain (AD) vector pGADT7-Rec (Clontech, Mountain View, CA) was mated with yeast strain AH109 transformed with pGBKT7-GIP and the diploids screened for GIP-interacting clones using standard methods [12]. Clones capable of activating all four integrated reporter genes: *HIS3*, *ADE2*, *MEL1* and *LacZ* under the control of the Gal4-responsive upstream activating sequence (*UAS*) were identified by plating transformants on selective solid media (SD/-Ade/-His/-Leu/-Trp/X-α-gal) (Clontech, Mountain View, CA). Transformants were subsequently checked for beta-galactosidase expression from the *lacZ* reporter gene [12]. *Escherichia coli* was transformed with the plasmids isolated from positive yeast clones. Plasmids isolated from these bacterial transformants were tested again in yeast to confirm their ability to activate the reporter genes when co-transformed with pGBKT7-GIP. GIP bait-dependent reporter gene expression was assessed by transforming two-hybrid reporter yeast with plasmids expressing the putative GIP-interactors in the absence of other fusion proteins and with other unrelated fusion proteins (data not shown). cDNA inserts were analysed by *Hind*III and *Hae*III digestion, using the Insert Check PCR Kit (Clontech, Mountain View, CA) and by DNA sequencing (J.P.Robarts Research Institute, London, Ont.).

A second independent cDNA library was used for confirmation experiments (Clontech, Mountain View, CA). Primers designed to verify the presence of the BAI2 C-terminal encoding region in the library plasmid were based on the published sequence (Genbank accession number; NM_001703.2); 5'-ggcgaattcgggaacatgctcatcgaatc-3' (forward) and 5'-catactcgagactcacacctgtctgtgaag-3' (reverse), giving a band of 1485 bp upon PCR amplification. Polymerase chain reactions were carried out as described and reaction products were analysed on 1% agarose gels (5 V/cm) in TBE buffer [12]. Gels were photographed under UV light after staining with ethidium bromide (0.5 µg/mL). The expression of GIP and BAI2 fusion proteins was examined by Western blot using either anti-Gal4 DNA-BD or anti-Gal4 DNA-AD mouse mono-clonal antibodies (Clontech, Mountain View, CA).

2.3. Expression and purification of ¹⁵N- and unlabeled GIP

GIP protein was expressed in *E.coli* and purified as previously described [13], *E.coli* (strain BL21DE3pLysS) was transformed with plasmid pET-3c/GIP and cells were cultured in M9 minimal media containing ¹⁵N-labeled ammonium chloride for ¹⁵N-labeled GIP. The expressed protein was purified in a single step using size exclusion chromatography [13].

2.4. Fluorescence

All fluorescence spectra were recorded on a PerkinElmer Precisely LS 55 Luminescence spectrofluorometer at 25 °C (λ_{ex} 280 nm). Emission spectra were recorded over a range of 300–500 nm with 1 nm steps. The peptide (RDGDFQTEV-COOH) was obtained with >95% purity from Chi Scientific (MA). Titration experiments were done as previously described [13].

2.5. Circular Dichroism (CD)

All circular dichroism (CD) experiments were performed on a Jasco J-810 automatic recording spectropolarimeter. Far-UV CD spectra were measured in a 0.05 cm quartz cell at room temperature. The buffer used was 20 mM phosphate buffer (pH 6.5). The protein concentration was 30 μM . Data were averaged over 100 scans for each protein sample and over 50 scans for each control sample. Response time was 1 s, and scan speed was 100 nm min^{-1} .

2.6. Nuclear Magnetic Resonance (NMR)

All NMR data were collected at 298 K on a Bruker Avance 600 MHz spectrometer equipped with a triple resonance H/C/N TCI cryoprobe at the Department of Chemistry and Biochemistry, Auburn University, Auburn, AL. The data were processed using NMRPipe and analyzed using Sparky. The interaction study was carried out by titration of 100 μM ^{15}N -labeled GIP with the BAI2 peptide. The amide chemical shift perturbations ($\Delta\delta$) were calculated as $\Delta\delta = \sqrt{\Delta\delta^2} = \sqrt{\{[\Delta\delta^{15}\text{N}/10]^2 + [\Delta\delta^1\text{H}]^2\}}$. In the equation, $\Delta\delta^{15}\text{N}$ was divided by 10 to account for the difference in the gyromagnetic ratio of the ^{15}N and ^1H nuclei to give roughly equal weighting for both types of chemical shift changes. The program ModelTitr was used to calculate the dissociation constant values for various residues of GIP.

3. Results and Discussion

3.1. Yeast Two Hybrid Screening

Yeast two-hybrid assays have become a valuable approach for identifying protein-protein interactions. This method detects *in vivo* interactions and requires no prior knowledge or purification of potential interacting partner proteins. In order to identify proteins interacting with GIP, a plasmid expressing GIP fused to the yeast Gal4 DNA binding domain (Gal4_{BD}), pGBKT7-GIP, was constructed. The expression of Gal4_{BD}-GIP in yeast was confirmed with an antibody specific for Gal4_{BD}, which detected one major species with a mobility close to the 35 kDa expected for the Gal4_{BD}-GIP fusion protein (Fig. 1A, lane 1). The size of the fusion protein was larger than that of Gal4_{BD} expressed in yeast from the vector pGBKT7 (Fig. 1A, lane 2). Neither of these proteins were detected in non-transformed cell lysates (Fig. 1A, lane 3).

The expression of the Gal4_{BD}-GIP fusion protein was not toxic in yeast as shown by plating the cells transformed with either empty pGBKT7 vector or pGBKT7-GIP on SD medium lacking tryptophan to select for the presence of the plasmid. No significant difference in the number or size of the colonies between the two populations of transformed cells were observed indicating that GIP expression was not toxic (data not shown).

The Gal4_{BD}-GIP fusion protein was also checked for autoactivation. When yeast strain AH109 was transformed solely with pGBKT7-GIP, the yeast could grow on SD medium lacking tryptophan but did not grow when adenine and histidine were also omitted. This indicates that the Gal4_{BD}-GIP bait protein did not activate the reporter genes in the absence of an interacting partner (data not shown).

We used yeast transformed with the Gal4_{BD}-GIP-expressing bait plasmid to screen a human fetal brain cDNA Gal4_{AD} library. Library titering and mating efficiency were calculated as 2.3×10^8 and 10%, respectively. Screening 1.06×10^7 Y187 clones, we isolated several transformants that were able to form colonies under conditions that required Gal4-responsive reporter gene activation suggesting they had received a library plasmid expressing a GIP-interacting protein. Further tests for expression of the other reporter genes reduced the number of putative positive clones. Library plasmids were isolated from the yeast transformants and the cDNA insertions were characterized by restriction mapping and sequencing. A BLASTN search of the Genbank DNA sequence database with these sequences identified Brain-specific angiogenesis inhibitor 2 (BAI2) as one of the positive clones. Based on the published sequence, the library plasmid encoded the C-terminal 200 amino acids of the BAI2 protein (Genbank accession number: NM_001703.2). To verify that the isolated library plasmid was expressing a Gal4AD-BAI2 fusion protein of predicted size, protein was extracted from the yeast transformant and analyzed by Western blotting using an anti-HA antibody that recognizes an HA epitope encoded by the pGADT7-Rec vector that is incorporated in all fusion proteins expressed by the cDNA library plasmids (Fig. 1B). A single species, approximately 20 kDa larger than HA epitope-tagged Gal4AD (Fig. 1B, lane 2) was detected in yeast transformed with the BAI2-encoding library plasmid (Fig. 1B, lane 1) but not in untransformed yeast (Fig. 1B, lane 3). The results indicate the Gal4AD-BAI2 fusion protein should terminate with the normal BAI2 C-terminal amino acid residues which would be available for interaction in the yeast with the PDZ domain in Gal4BD-GIP.

Sequence analysis showed that the amino acid residues at the C-terminal end of BAI2 are T-E-V, which is a class I PDZ-domain recognition motif. To characterize the potential interaction between GIP and BAI2 by biophysical approaches, an eight amino acid residue BAI2 peptide (RDGDFQTEV-COOH) was synthesized as a surrogate for the C-terminus of BAI2. It has been shown that C-terminal peptides can be utilized as surrogates for the target proteins to efficiently mimic this type of protein-protein interaction [13,14].

3.2. Interaction of Target Peptide and GIP by Fluorescence Spectroscopy

When the peptide was titrated against unlabeled GIP, it showed a small but consistent decrease in fluorescence intensity (Fig. 2A). The dissociation constant K_D ($K_D = 1/K_a$) was determined using the OriginPro 6.1 software. The decrease in the fluorescence intensity was calculated as $(F_0 - F_C)/(F_0 - F_{\min})$, where F_0 is the initial fluorescence intensity of free GIP; F_C is the corrected fluorescence intensity at a ligand concentration $[C]$, and $F_{\min}$ is the fluorescence intensity at the saturating concentration of the peptide. The data were fitted to a nonlinear regression of the plot of $(F_0 - F_C)/(F_0 - F_{\min})$ against $[C]$ with the equation corresponding to a single binding site. The titration of the BAI2 peptide with GIP yielded a dissociation constant of 0.71 μM (Fig. 2A). To determine the thermodynamic nature of the interaction, the free energy change of the association was calculated using the following equation: $\Delta G = -RT \ln K_a$, where K_a is the association constant, T is temperature and R is universal gas constant. By putting the experimentally determined K_a ($K_a = 1/K_D$) value into this equation, the ΔG value for binding of the BAI2 peptide to GIP is calculated to be 35.08 kJ mol^{-1} , which reflects the spontaneous binding of the peptide to GIP.

3.3. CD Analysis of Peptide Binding Interaction

Using CD analysis, the secondary structure of GIP showed significant changes with the titration of different concentrations of the peptide (Fig. 2B) Contribution from the peptide/buffer was subtracted from the CD spectrum. CD data of the GIP-peptide complex was deconvoluted using the program CDPPro and the secondary structure content was calculated. The helix content was found to be reduced by $\sim 47\%$, random coil content by $\sim 8\%$ and the

β -sheet structure content increased by ~ 29%. Although, the increase in β -sheet content in all these cases can be explained by the mode of binding of these peptides to the GIP through β -strand addition, closer examination of the representative complex structure of GIP with its binding partner does not show any change in the helical content but does indicate some displacement of the helical structure in space [7, 9].

3.4. Interaction of GIP with the peptide by $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC NMR

To examine the interaction of GIP with the C-terminal BAI2 peptide more thoroughly, NMR was employed. For the investigation of binding, ^{15}N -labeled GIP protein was titrated with the synthetic BAI2 peptide to excess (~60 times that of the protein) until complete saturation was achieved. During the course of the titration, the fingerprint region of the protein in the 2D $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC spectra was monitored (Fig. 3). From the overlay, it is evident that most of the residues of GIP show some changes in chemical shifts upon binding with the peptide, while some residues show more dramatic changes. Using the program ModelTitr, the dissociation constant (K_D) values for various residues of GIP were calculated (Table I) by non-linear least-squares fitting of the chemical shift data against ligand concentration to the Langmuir isotherm that involves the assumption of a stoichiometry of 1:1 between the ligand and the protein (*i.e.* one binding site). The dilution effect on the concentration of the protein due to the addition of the ligand was corrected in the program. The calculated dissociation constant (K_D) value from NMR technique (97.77 μM on an average) is different from the value obtained from fluorescence technique. Since the dissociation constant (K_D) value varies depending upon techniques and initial protein concentration used [13], such a difference in the K_D values obtained from two different techniques is acceptable. From the K_D values of both fluorescence and NMR techniques, the dissociation constant (K_D) value falls in the range of low to mid μM , which indicates a moderate affinity of GIP for the BAI2 peptide.

3.5. Chemical Shift Perturbation of GIP upon Binding to the Peptide

Mapping the chemical shift perturbation with respect to residue number for a protein is a way to demonstrate the putative interacting portions of a protein with its interacting partner. For the mapping study of GIP with BAI2 peptide, a series of the 2D $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC spectra of GIP with increasing peptide concentrations were analyzed. The chemical shifts of most of the residues of GIP in both free and complex forms were determined. During analysis of the 2D $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC spectra, the amide proton and nitrogen resonances of most residues showed gradual shifts with increasing peptide concentration, indicating that the complex was mostly in the fast exchange regime on the NMR time scale. However, some residues disappear or decrease in intensity below the noise level threshold with increasing peptide concentrations but reappear at higher peptide concentrations suggesting that these residues were in intermediate exchange on the NMR time scale. For example, Leu 29 and Gly 30 initially disappear with increasing peptide concentrations but reappear at high peptide concentrations. Some of the residues could not be characterized for this mapping study because of the complete absence of the peak from the HSQC spectrum or peak overlapping. These include Met 1, all five proline residues, Val 12, Leu 21, Phe 31, Glu 48, Lys 50, Val 57, Val 80 and Val 105.

Residues that constitute the $\beta 2$ strand (residues 31 to 35) and the $\alpha 2$ helix (residues 90 to 97) of the protein show the most chemical shift perturbations compared to other residues as seen on the 2D HSQC spectrum and mapping of chemical shift perturbations (Fig. 3 and Fig. 4). This observation is consistent with that of interaction of GIP with a canonical C-terminal binding motif recognition peptide [9,13]. Most of the residues located within this region show greater than 0.1 ppm perturbations except residues Gln 92, Ala 93 and Leu 97 (Fig. 4). The large perturbations occur because the peptide directly interacts with most of these

residues of the β 2 strand and α 2 helix. Residues Leu29 and Gly30 show very large perturbations (greater than 1.0 ppm) (Fig. 4) probably due to the hydrogen bonding formed between these two residues and the C-terminal end of the peptide. Such large chemical shift perturbations for Leu29 and Gly30 are reminiscent of our previous work on the interaction of GIP with a C-terminal peptide analog of Glutaminase L that was reported recently [9]. Also, another cluster of residues showing prominent perturbations are residues 66 to residues 71 that form the α 1 helix of the protein (Fig. 4). Within this region, residues Ala 66, Glu 67, Ile 68 and Ala 69 show greater perturbations (greater than 0.1 ppm). The significant changes in chemical shifts of this region (α 1 helix) of the protein are not due to the direct interaction with the peptide but rather due to the change in the surrounding environment of the helix since this helix is in close proximity to the binding pocket of the protein. In a recent report, several long-range NOEs were observed between Ile 28 and the α 1 helix indicating a close spatial proximity between the β a- β b loop and the α 1 helix for the free state of the protein but only a very few NOEs were present for that region of the complex form of the protein with Glutaminase L peptide (BMRB entry: 17254 and 17255) [9]. Thus, the reason for comparatively higher chemical shift perturbation for residue Ile 28 (greater than 0.5 ppm) (Fig. 4) could be twofold. First it is very close to the binding pocket. Second the binding of the BAI2 peptide to the protein probably results in the disruption of the interaction (NOEs) between residue Ile 28 and α 1 helix. Although there are certain pockets of residues that show significant chemical shift perturbations, the binding of the peptide to the protein seems to induce a change in the chemical environment over nearly the entire protein except for the termini. The N- and C-termini of the protein do not show any significant changes in the chemical shifts (Fig. 4) upon peptide binding. Thus, the mode of BAI2 peptide binding to GIP can be characterized as allosterically driven analogous to the binding of the Glutaminase L peptide to GIP [9].

BAI2 is a member of the adhesion-G protein-coupled receptors (GPCRs) [15, 16]. It is composed of 521-amino acids and mainly expressed in neurons [17]. BAI2 possesses a Src homology 3 (SH3) domain, composed of 50–60 amino acids that mediates protein-protein interactions and was previously reported as interacting with the C-terminus of Brain-Specific Angiogenesis Inhibitor 1 (BAI1) via its SH3 domain as shown by *in vitro* binding assays [17]. This is the first study reporting an interaction between BAI2 and GIP with an extensive biophysical characterization of their interaction.

Brain-specific Angiogenesis Inhibitor 2 (BAI2) is a new partner protein for GIP. > BAI2 interaction with GIP was revealed by yeast two-hybrid assay. > Binding of BAI2 to GIP was characterized by NMR, CD and fluorescence. > BAI2 and GIP binding was mediated through the C-terminus of BAI2.

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Fig. 1.

Expressional analyses of bait and prey proteins. A. AH109, transformed with pGBKT7-GIP expressing GIP as a Gal4_{BD}-fusion protein (lane 1) or the Gal4_{BD} vector pGBKT7 (lane 2) or that was untransformed (lane 3) was cultured. (B) AH109, transformed with library plasmid pGADT7-BAI2 (lane 1) or pGADT7 (lane 2), or untransformed (lane 3), was cultured. Total cell lysates were analysed by 10% SDS -PAGE and Western blotting with either mouse monoclonal anti-Gal4_{BD} (A) or anti- Gal4_{AD} (B) antibodies.

Fig. 2.
 (A) Fluorescence emission spectrum of GIP with the BAI2 peptide. Non-linear curve fitting plotting $(F_0 - F_C)/(F_0 - F_{\min})$ versus peptide concentration. Inset represents fluorescence emission plots corresponding to (top to bottom) 0 to 20 μM concentration of the peptide to 1 μM protein sample. Black arrow indicates the quenching of fluorescence of GIP upon peptide binding in a downward fashion. (B) Changes in the CD spectra of GIP upon binding to increasing concentrations of the peptide. The protein to peptide ratios for the corresponding color codes are indicated in the legend.

Fig. 3.

Changes of 2D $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC spectrum upon addition BAI2 peptide to 100 μM of ^{15}N -labeled GIP. (A) The 2D $\{^1\text{H}, ^{15}\text{N}\}$ -HSQC spectra demonstrating chemical shift perturbations of residues upon titration of the peptide to GIP. (B) Expanded regions of the HSQC spectra for residue E17. Ratios of GIP to the peptide are 1:0 (green), 1:0.2 (tomato), 1:0.4 (blue), 1:0.6 (beige), 1:0.8 (turquoise), 1:1 (gold), 1:2 (coral), 1:3 (purple), 1:5 (maroon), 1:7 (orange), 1:10 (red), 1:20 (cyan), 1:40 (white), 1:60 (magenta). (C) The NMR titration binding curve for residue E17 of GIP with the peptide titration. The determined K_D value is $64.8 \pm 10.6\%$ μM .

Fig. 4.
Chemical shift perturbations ($\Delta\delta$) of the GIP backbone amide groups upon binding with the peptide.

Table I

Dissociation constants of various residues of GIP upon binding with the peptide by NMR

Interaction with RDGDFQTEV	
Residues of GIP	Dissociation constants, μM
E17	$64.78 \pm 10.64\%$
R22	$101 \pm 5.6\%$
D38	$92.5 \pm 3\%$
F46	$137.7 \pm 7.57\%$
E62	$102.3 \pm 5.27\%$
A66	$86.58 \pm 2.20\%$
L71	$85.85 \pm 7.82\%$
N81	$84.28 \pm 7.29\%$
T86	$104.3 \pm 6.46\%$
E102	$118.4 \pm 6.26\%$