Journal of Biomolecular NMR **28:** 409–410, 2004.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of human RGSZ1

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Received 25 July 2003; Accepted 30 September 2003

Key words: assignments, GPCRs, G protein signaling, RGSZ1, secondary structure

Biological context

The regulator of G protein signaling (RGS). These proteins play a key role in modulating the G-protein activity. The RGS family contains more than 20 proteins and have been categorized into five subfamilies (Ross and Wilkie, 2000). However regulation of specific Gα subtypes, between the RGS proteins has been demonstrated in vitro and is likely due to subtle sequence differences. For example, RGS2 is a more effective GAP for Gαq, while RGS4 can activate both GαI and Gαq. RGSZ1 was initially identified to exclusively activate Gαz. Recent work has confirmed that RGSZ1 is also an effective GAP for GαI (Wang et al., 2002). As a 20 member family, RGS proteins are widely expressed, with some tissues expressing multiple mRNA transcripts for the RGS subtypes. For example, RGSZ1 is exclusively expressed in the brain (Wang et al., 1997); whereas RGS4, which is highly expressed in the frontal cortex (Gold et al., 1997), and also in other tissues. Despite being similar in size (approximately 150 amino acids); RGSZ1 (Glick et al., 1998) shares 45% sequence identity with RGS4. The solution structure of RGS4 (Moy et al., 2000) and X-ray structure of Gα bound RGS4 (Tesmer et al., 1997) have been determined. To further understand the structure/function and specificity relation of RGSZ1, we have begun a structural study and we report here the ^{1}H, ^{13}C, and ^{15}N NMR assignments and secondary structure of RGSZ1. These data provide a basis for determining the solution structure of free RGSZ1 and for further investigations in design of novel therapeutics involving the RGS and Gα interactions in the regulation of GPCRs signal transduction.

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Methods and results

RGSZ1 (amino acids 75-217) (Glick et al., 1998) with carboxyl terminal 6X His tag in pET21a (Novagen) was expressed from *E. coli* strain BL21(DE3)-RP in minimal media supplemented with ^{13}C-glucose (2g/L) and ^{15}N ammonium sulfate (2g/L). The cells were resuspended in 20 mM Tris pH 8.0, 500 mM NaCI, and 5 mM imidazole. After disruption with a microfluidizer and centrifugation, the supernatant was applied to a column of Nickle-NTA agarose. The RGSZ1 protein was eluted with a gradient of 5 mM to 150 mM imidazole, and then loaded onto a TSK gel G3000sw column, equilibrated with a buffer of 50 mM ammonium bicarbonate pH 7.5 and 1 mM EDTA. Fractions containing RGSZ1 were applied onto a DEAE-Sepharose column then eluted with 120 mM NaCI. The yield of pure RGSZ1 was 2 mg/gram of cell pellet as measured with an extinction coefficient of 17,810 M^{-1} cm^{-1}.

The NMR samples contained 0.4 mM ^{15}N/^{13}C labeled RGSZ1 in a buffer containing 100 mM sodium phosphate, 3 mM NaN3, pH = 6.8 in 90% H_2O/10% D_2O. Spectra were processed using the NMRPipe software package and analyzed with PIPP. The ^{1}H, ^{15}N, and ^{13}C assignments were obtained utilizing the following experiments, CBCA(CO)NH, CBCANH, CC(CO)NH, HBHA(CO)NH, HNCO and

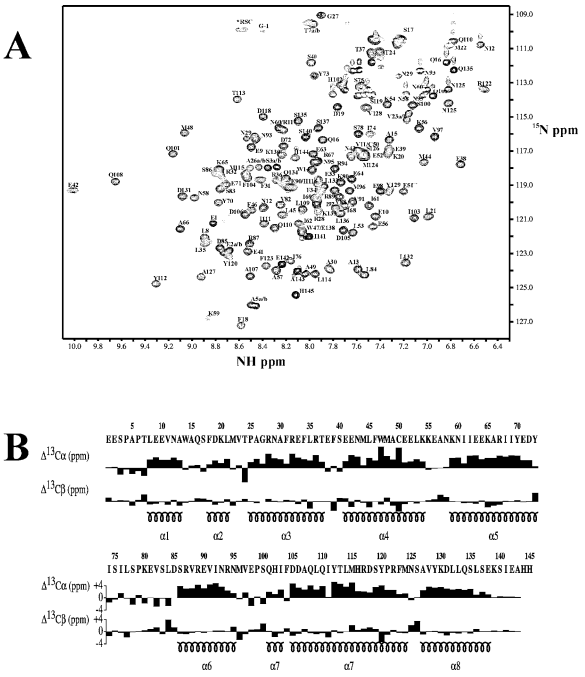

Figure 1. (A) 600 MHZ MHz ^{1}H-^{15}N-HSQC spectrum of ^{15}N/^{13}C -labeled RGSZ1 at 25 °C in 100 mM sodium phosphate, 3 mM NaN_3, pH = 6.8 buffer. The resonance assignments are listed next to the crosspeaks. Multiple assignments are labeled for residues between E1 and A5 and between residues M22 and A26 attributed to cis-trans isomerization. Tryptophan indoles were not included in the spectrum. (B) Summary of the the 13Cα and 13Cβ secondary chemical shifts observed for RGSZ1 withand the secondary structure. deduced from this data.

HNHA (Moy et al., 2000). Some ambiguous assignments were further confirmed by sequential and intra residue NOEs in the ^{15}N-edited NOESY-HSQC and CNH-NOESY spectra (Diercks et al., 1999). ^{1}H chemical shifts were referenced to external TSP (0.00 ppm) in D_2O, while ^{15}N and ^{13}C chemical shifts were referenced indirectly to the absolute frequency ratios ^{15}N/^{1}H=0.101329118 and ^{13}C/^{1}H=0.251449530.

The secondary structure of RGSZ1 is based on results from the program TALOS (Cornilescu et al., 1999). RGSZ1 is composed of eight helical regions corresponding to residues 8–13 (α1), 18–21 (α2), 25–37 (α3), 41–54 (α4), 59–73 (α5), 86–95 (α6), 101–124 (α7) and 127–138 (α8).

Extent of assignments and data deposition

^{15}N and amide assignments were obtained for all residues except L77 and E81 which are located in the long flexible loop region between α5 and α6. RGSZ1 contains multiple proline residues (P4, P6, P79, P99, and P121) which prohibited complete assignment of side chains of those residues preceding proline utilizing HSQC-related triple resonance experiments. We are missing some ^{13}C side chain assignments for some residues containing long side chains such as C_ε of K20 and K54 and $C_{\delta 1}$ of I68, I69, and I111. The aromatic rings of phenylalanine, tyrosine, and tryptophan have not been assigned. The Hα and Hβ for Thr and Ser residues were unambiguously assigned for utilizing the HNHA experiment. Two species are present at the N-terminus due to *cis/trans* isomerization of Pro4. This is also observed between helices 2 and 3 due to the *cis/trans* isomerization of Pro25. In both cases the trans isomer is the major form. Figure 1A shows the ^{1}H-^{15}N HSQC spectrum of RGSZ1. Backbone and side chain NH assignments are labeled on the spectrum. A summary of the secondary Cα and Cβ shifts along with the secondary structure as a function of residue number are shown in Figure 1B. Assignments described herein have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB number 5872.

Acknowledgements

We thank Drs Robert Powers and Mark Stahl for valuable discussions and suggestions during the course of this work.

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