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# Letter to the Editor: $^{1}$H, $^{15}$N, $^{13}$C, and $^{13}$CO assignments and secondary structure determination of RGS4*

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## Biological context

Cellular signal transduction involves the agonist-induced conformational change to a heterotrimeric guanine nucleotide-binding protein (G-protein) coupled to a cell surface receptor (for review see Neer, 1995). This conformational change in the G-protein causes its Gα subunit to release GDP and bind GTP. The association of Gα with GTP results in a modification in the structure of the three 'switch' regions which facilitates dissociation of Gα from Gβγ. The released subunits are then available to interact with a variety of target proteins to elicit the desired response. Inactivation of the signal occurs from a reversal of the process. The GTP bound to Gα is hydrolyzed, which results in the re-association of Gα with Gβγ.

A number of factors have been identified which affect the intensity and duration of the signal cascade. Of particular interest are the regulators of G-protein signaling (RGS) which affect the rate of GTP hydrolysis by binding to the Gα subunit (for reviews see Dohlman and Thorner, 1997; Arshavsky and Pugh, 1998), where an X-ray structure of RGS4 bound to $G_{i\alpha_1}$ has been previously reported (Tesmer et al., 1997). Thus, RGS act as negative regulators or attenuators of the G-protein induced signal. Members of the RGS family have region-specific expression in the brain where RGS4 is perhaps the most widely distributed and highly expressed RGS subtype. The regulation of RGS expression has been correlated with a response to a seizure and may suggest an adaptive response in the brain signal transduction pathway to compensate for desensitization and sensitization of G-protein-coupled receptor function. Here we report the near complete $^{1}$H, $^{15}$N, $^{13}$CO, and $^{13}$C NMR assignments and secondary structure of free RGS4. These data provide a basis for determining the solution structure of free RGS4 and for further investigation in the design of novel RGS4 inhibitors.

## Methods and results

The uniform $^{15}$N- and $^{13}$C-labeled 166 amino acid core domain of RGS4 with a C-terminal polyhistidine tag (6 His) was expressed in *E. coli* strain BL21(DE3) containing the plasmid pRGS4. The RGS4 protein was purified using affinity chromatography on a $Ni^{2+}$ column and purified to homogeneity (>95%) following ion exchange chromatography on Resource S at pH 5.5. N-terminal amino acid sequencing was performed to confirm the protein's identity while the uniform $^{15}$N and $^{13}$C labeling of RGS4 was confirmed by MALDI-TOF mass spectrometry (PerSeptive Biosystems). The NMR samples contained 1 mM of RGS4 in a buffer containing 50 mM potassium phosphate, 2 mM $NaN_3$, 50 mM deuterated DTT, in either 90% $H_2O$/10% $D_2O$ or 100% $D_2O$ at pH 6.0. All NMR spectra were recorded at 30 °C on a Bruker AMX-2 600 spectrometer equipped with a triple-resonance gradient probe. Spectra were processed using the NMRPipe software package (Delaglio et al., 1995) and analyzed with PIPP (Garrett et al., 1991), NMRPipe and PEAK-SORT, an in-house software package.

*Supplementary material available from the authors: a table containing the $^{1}$H, $^{15}$N, $^{13}$CO, and $^{13}$C resonance assignments for RGS4.

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The assignments of the $^{1}H$, $^{15}N$, $^{13}CO$, and $^{13}C$ resonances were based on the following experiments: CBCA(CO)NH, CBCANH, C(CO)NH, HC(CO)NH, HBHA(CO)NH, HNCO, HCACO, HNHA, HNCA, HCCH-COSY and HCCH-TOCSY (for reviews see Bax et al., 1994; Clore and Gronenborn, 1994). The accuracy of the RGS4 NMR assignments was further confirmed by sequential NOEs in the $^{15}N$-edited NOESY-HMQC spectra. Given the fact that the RGS4 structure is exclusively α-helical, the sequential $NH_i$-$NH_{i+1}$ NOEs were extremely useful in completing the RGS4 backbone assignments.

The secondary structure of the RGS4 is based on characteristic NOE data involving the NH, Hα and Hβ protons from $^{15}N$-edited NOESY-HMQC and $^{13}C$-edited NOESY-HMQC spectra, $^{3}J_{HN\alpha}$ coupling constants from HNHA, slowly exchanging NH protons and $^{13}C\alpha$ and $^{13}C\beta$ secondary chemical shifts (for reviews see Wüthrich, 1986; Wishart and Sykes, 1994). It was determined that the RGS4 is composed of seven helical regions corresponding to residues 7–12 ($\alpha_1$); 17–36 ($\alpha_2$); 40–53 ($\alpha_3$); 61–71 ($\alpha_4$); 86–95 ($\alpha_5$); 105–125 ($\alpha_6$) and 128–132 ($\alpha_7$). The RGS4 overall fold essentially comprises two four-helix bundles where the long helical region $\alpha_6$ is part of both bundles. A distinct difference in the RGS4 secondary structure at the C-terminus was unexpectedly observed between the NMR structure of free RGS4 and the $G_{i\alpha_1}$-RGS4 X-ray structure. The RGS4 X-ray structure indicates that residues 104–116 and 119–129 are helical where only residues V5 to T132 are observed. This compares with the NMR structure, where residues 105–125 and 128–132 are helical and residues P134 to H166 appear to be extremely mobile because of the sharp linewidths. This suggests a conformational change in RGS4 upon binding $G_{i\alpha_1}$.

## Extent of assignments and data deposition

The backbone $^{1}H$, $^{15}N$, $^{13}C$, and $^{13}CO$ assignments are essentially complete for RGS4. The native sequence for RGS4 was appended with six histidines at the C-terminus to aid in purification of the protein. Interestingly, the last five histidines and C-terminal proline were the only unassigned residues in the protein. Since it is not uncommon for a protein the size of RGS4 to contain a few short stretches with incomplete assignments, which is usually attributed to mobile regions of the protein, obtaining the complete assignments for RGS4 implies a well-packed ordered structure. The side chain assignments are nearly complete where a majority of the missing information occurs in residues with long side chains which are potentially solvent exposed. Figure 1 shows representative strips from the $^{15}N$-NOESY-HMQC spectra for residues L34 to I43, corresponding to part of α-helical regions 2 and 3, indicating both sequential and intraresidue assignments. A table of chemical shifts has been deposited in the BioMagResBank Database (accession code 4386).

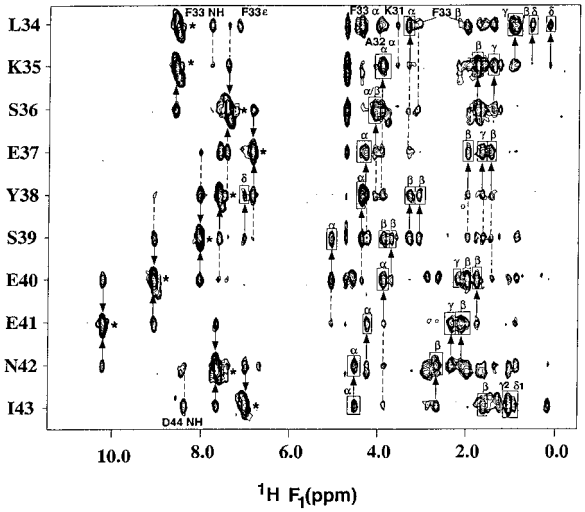


*Figure 1.* Composite of amide strips taken from the 100 ms mixing time 3D $^{15}N$-edited NOESY-HMQC spectrum of the RGS4 complex for the stretch of residues from Leu-34 to Ile-43 corresponding to part of $\alpha_B$. The diagonal peaks are indicated by an asterisk, NH(i)-NH(i±1), NH(i+1)-Hα, and NH(i)-NH(i±1) and NH(i+1)-Hβ(i) NOEs are indicated by solid arrows, and NH(i+2,3,4)-Hα(i), and NH(i+2,3,4)-Hβ(i) NOEs are indicated by dashed lines. Intraresidue NOEs are boxed and labeled.

## References


Arshavsky, V.Y. and Pugh Jr., E.N. (1998) *Neuron*, **20**, 11–14.

Bax, A., Vuister, G.W., Grzesiek, S. and Delaglio, F. (1994) *Methods Enzymol.*, **239**, 79–105.

Clore, G.M. and Gronenborn, A.M. (1994) *Methods Enzymol.*, **239**, 349–362.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Dohlman, H.G. and Thorner, J. (1997) *J. Biol. Chem.*, **272**, 3871–3874.

Garrett, D.S., Powers, R., Gronenborn, A.M. and Clore, G.M. (1991) *J. Magn. Reson.*, **95**, 214–220.

Neer, E.J. (1995) *Cell*, **80**, 249–257.

Tesmer, J.J.G., Berman, D.M., Gilman, A.G. and Sprang, S.R. (1997) *Cell*, **89**, 251–261.

Wishart, D.S. and Sykes, B.D. (1994) *Methods Enzymol.*, **239**.

Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York, NY.