Evidence for the Dimerization of Human Regulator of G-protein Signalling 5 (RGS5)

Zhao Yang^a, Sabrina Gaudio^a, Wei Song^b, Matthew Greenwood^b,
Gaël Jean-Baptiste^b and Michael T. Greenwood^{a,b}

The ^aDepartment of Anatomy and Cell Biology and the ^bDepartment of Medicine, McGill University, Montreal, Quebec

Key Words

RGS • RGS-GFP • RGS dimmer • Yeast • Heterologous expression

Abstract

RGS5 is a R4 type RGS that regulates GPCR signalling. Using western blot, we detected RGS5 as a specific 23 kDa protein in cells overexpressing RGS5. A 42 kDa band representing a possible RGS5 dimer was also detected. Given that GPCRs and their associated proteins form complexes involving multiple protein-protein interactions, we investigated the possibility that the 42 kDa band represents an RGS5-RGS5 dimer. RGS5 dimerization was confirmed by the analysis of a GFP tagged RGS5 fusion in yeast and with two-hybrid assays. Analysis of RGS5 in HEK293A cells suggests that the dimer may serve a regulatory function since it is longer lived than the monomer.

Introduction

Regulators of G-protein Signalling (RGS) refers to a conserved 120-125 residue motif that was first identified for its ability to negatively regulate G-Protein Coupled Receptor (GPCR) signalling [1-3]. There are now well over 25 RGS containing mammalian proteins that are categorized into several distinct sub-families [4-6]. Many of these RGSs are large proteins that also harbour other functional motifs such as PDZ, PH and DEP domains [7]. This implies that these RGSs interact with multiple protein partners. On the other hand, members of the R4 subfamily of RGSs are small proteins (ca. 200 residues) that contain little more than an RGS motif [4-6]. The RGS box from most of these R4 RGSs have been shown to be capable of inhibiting GPCR signalling by acting as a GTPase Activating Protein (GAP) for receptor activated

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Michael T. Greenwood, Dr.
Polypeptide Laboratory, McGill University
3640 University St., room W315, Montreal, Quebec H3A 2B2 (Canada)
Tel. +1 514 398-6783, Fax +1 514 398-3923
E-Mail michael.greenwood@mcgill.ca

GTP-bound G_{α} subunits. There appears to be distinct but overlapping specificity of the different RGSs for the different G_{α} -proteins [5,7,8]. In addition, most of the evidence suggests that the R4 RGSs cannot act as a GAP for $G_{\alpha s}$ subunits. Nevertheless, a number of these RGSs can block signalling from $G_{\alpha s}$ coupled GPCRs by acting as an effector antagonist and effectively blocking $G_{\alpha s}$ mediated activation of effectors such as adenylyl cyclase [9]. Individual R4 RGSs also show distinct receptor specific preferences in their ability to inhibit signalling from different GPCRs [10]. This specificity appears to be determined by the ability of the different R4 RGSs to either bind to specific subsets of GPCRs or to bind to specific GPCR binding adapter proteins [11]. In spite of the absence of definite recognizable motifs, the N-terminal non-RGS portions of these proteins have been implicated in mediating their receptor specificity and their ability to function as effector antagonists [11, 12]. Thus although R4 RGSs are small proteins, their ability to interact with a number of other proteins in addition to their interaction with G_{α} subunits is critical for their function as modulators of GPCR signalling [13].

Multiple protein-protein interactions are a common theme for most signal transduction cascades including GPCRs [14]. In effect, GPCRs and their associated proteins are now known to assemble into large protein complexes [15, 16]. In addition to interacting with a wide range of different proteins, GPCRs also form homo- and hetero-oligomers [17]. Although RGSs are members of the GPCR containing complexes, the entire repertoire of proteins that are capable of interacting with the R4 RGSs is not known [11, 13].

We have previously generated specific RGS5 anti-serum and we have shown that this protein migrates as 23 kDa protein that is abundantly expressed in cardiac and skeletal muscle [18]. Here we continue our analysis of the RGS5 protein and we report a new binding partner for RGS5. In effect, we demonstrate that RGS5 can be detected as a dimer by western blot analysis in both yeast and cultured mammalian HEK293A cells that overexpress RGS5. Using a series of other experimental approaches including the expression and analysis of GFP tagged RGS5 in yeast as well as two hybrid assays, we provide evidence that RGS5 does indeed interact with itself. We also demonstrate that formation of the RGS5 dimer may have a regulatory role since we observe that the dimer is longer lived than the monomer. Thus our results suggest that the dimerization of R4 RGSs, like RGS5, may provide an additional layer of complexity involved in regulating GPCR signalling complexes.

Materials and Methods

Plasmids

To make the RGS5-GFP fusion, RGS5 was amplified using the following forward 5'-ACA CTA GTC AAA CAA TGT GCA AAG GAC TTG CAG CTT TG-3' and reverse 5'-AGA AGC TTG CTT GAT TAA CTC CTG ATA AAA TCT-3' oligos with p423GAL1 RGS5 as a template [19]. The PCR product was subcloned as a *Spe* I-*Hind* III fragment into p426GAL1-GFP [20] to generate plasmid p426GAL1-RGS5-GFP encoding an in-frame RGS5-GFP protein fusion. The plasmids encoding the different GPCR C-tails fused to GFP were constructed by first amplifying the receptor C-tails using reverse transcribed RNA that was obtained from mouse tissues (brain, testes or heart) and the following oligos: SST₅: forward: 5'-AAC TAG TGG CAA ACA ATG TCT GAC AAC TTC CGC CAG AGC-3', reverse: 5'-AGAAGC TTG CAG CTT GCT GGT CTG CAT AAG-3'; β_2 AR: forward: 5'-CTA GTC AAA CAA TGA GTC CAG ATT TCA GGA TTGC C-3', reverse: 5'-AAG CTT GCA GTG GCA GGT CAT TTG TAC TAC A-3'; CB₁: forward: 5'-ACT GTC AAC AAT GAAGGA CCT GAG ACATGC TTTC CGC-3', reverse: 5'-AAG CTT GCA GAG CCT CGG CAG ACG TGT CTG T-3'; and LPA₄: forward: 5'-ACT AGT CAA ACA ATG TAC TTC ACT CTT GAA TCC TTT CAG-3', reverse: 5'-AAG CTT GGA AGG TGG ATT CCA GCA TTA A-3'. The amplified products were subcloned into p426GAL1-GFP as described above for RGS5. The yeast expression plasmid p425GAL-RGS5 and the mammalian expression plasmid for RGS5 were previously described [18, 19]. p426GAL1-ATG-GFP expressing GFP alone under the control of the galactose inducible *GAL1* promoter was previously described [20].

Yeast cells

The *Saccharomyces cerevisiae* strain BY4741-6055 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sst2Δ::G418^R*) was used for these studies (Research Genetics). Yeast cells were routinely grown on synthetic minimal media consisting of Yeast Nitrogen Base (YNB) containing 2% glucose supplemented with the appropriate amino acids and bases [21]. Plasmids were introduced into yeast using lithium chloride and the resultant transformants were selected and maintained by the omission of the appropriate supplements from the growth media (leucine for p425GAL1 and pACT2, uracil for p426GAL1, and tryptophan for pLexA-dir vectors). Glucose was replaced with 2% galactose and 2% raffinose to induce *GAL1* dependent expression. The semi-quantitative halo assay was used to assess the ability of RGS5-GFP to function as an RGS essentially as previously described [19, 22]. Sterile filter disks containing 1000 and 3000 pmoles of α -factor, were placed onto the top of a lawn of yeast cells and the plates were subsequently incubated at 30°C for 3 to 4 days.

Mammalian cultured cells

HEK 293A cells were grown in DMEM media containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (pen-strep). The cells were transfected with pcDNA alone or with pcDNA plasmid expressing RGS5 using lipofectamine (Invitrogen).

Protein extraction and western blot analysis

Soluble protein was extracted from yeast by directly heating the NaOH treated cells to 95°C in SDS-PAGE loading buffer as previously described [20]. Soluble protein was extracted from transiently transfected HEK293A cells using ice cold lysis buffer [23]. Equal amounts of soluble protein were separated by SDS-PAGE, transferred to nitrocellulose membrane and challenged with the previously described RGS5 anti-serum [18] or the commercially available GFP or LexA anti-sera (Santa Cruz Technology). HRP-conjugated secondary anti-serum were used and signals were subsequently detected with ECL plus (AmershamBioscience) followed by exposure to X-ray film (Kodak X-Omat). The relative expression levels were determined using Multi-Analyst software (BioRad). Unless otherwise indicated, western blots were repeated at least three times with similar results.

Two hybrid analysis

Plasmid pLexA-dir was used as the bait vector and plasmid pACT2 was used as the prey vector [24]. Human RGS5 was prepared for cloning into the prey vector by PCR amplification using the respective forward 5'-GCT TAC CCA TAC GAT GTT CCAGAT TAC GCT ATG TGC AAA GGA CTT GCA GCT-3 and reverse 5'-ACT TGC GGG GTT TTC AGT ATC TAC GAT TCT TAC TAC TTG ATT AAC TCC TGA TA-3' oligonucleotides. The N-terminal region of RGS5 was prepared for cloning into the bait vector by PCR amplification using the following respective forward 5'-GTT GGG GTT ATT CGCAAC GGC GAC TGG CTG ATG TGC AAA GGA CTT GCA GCT-3' and reverse 5'-TCA TAA GAA ATT CGC CCG GAA TAG CTG GCA GGG CCT CGT CCA GCG AGG T-3' oligonucleotides. The C-terminal RGS box regions of RGS5 was prepared for cloning into the bait vector by PCR amplification using the following respective forward 5'-GTT GGG GTT ATT CGCAAC GGC GAC TGG CTG CAG TGG CGT GAT TCC CTG GAC-3' and reverse 5'-TCA TAA GAA ATT CGC CCG GAA TTA GCT GGT TAC TAC TTG ATT AAC TCC TGA TA-3' oligonucleotides. The PCR products were subcloned by recombination in yeast using *EcoRI* digested two hybrid vectors [25]. Different combinations of the plasmids were transformed into the yeast strain DSY-1 (*MATa his3Δ200 trp1-901 leu2-3,112 ade2 LYS2::(lexAop)₄-HIS3 URA3::(lexAop)8-lacZ GAL4*). Freshly saturated cultures of the transformed were serially diluted and spotted onto YNB glucose agar plates with and without histidine [26].

Results

Identification of an RGS5 dimer

Human RGS5 is a 181 residue protein that belongs to the R4 RGS subfamily. It has a calculated molecular weight (MW) of 20.9 kDa and we have previously demonstrated that it migrates as a single band of 23 kDa when detected by western blot analysis [18]. The same 23 kDa band was detected in yeast and HEK293A cells overexpressing human RGS5 and in tissues endogenously

Fig. 1. Detection of a RGS5 dimer in cells overexpressing RGS5. (A) Western blot analysis of protein extracts prepared from control (Ctrl.) yeast cells or from yeast cells expressing RGS5 (RGS5). (B) Western blot analysis of protein extracts from HEK 293A cells. Cells were transfected with either the mammalian expression plasmid pcDNA (Ctrl.) or a pcDNA expressing a hRGS5 cDNA (RGS5). As a control, 0.5 µg of protein extract from yeast cells expressing hRGS5 was included on the gel (left lane). In both A and B, the extracts were challenged with RGS5 anti-serum. In addition the location of bands corresponding to the sizes of RGS5 and putative RGS5 dimers (dRGS5) are indicated. A non-specific band of approximately 87 kDa is apparent in the extracts prepared from both mammalian and yeast control cells and is indicated by an “*”. Similar results were obtained in four different experiments.

expressing RGS5. Here we demonstrate that in addition to recognizing the 23 kDa RGS5 protein, our affinity purified RGS5 anti-serum also recognizes proteins with apparent MWs of 87 and 42 kDa in extracts prepared from yeast cells expressing RGS5 (Fig. 1A). The 87 kDa band was also detected in extracts prepared from control yeast cells suggesting that it represents a non-specific

Fig. 2. Analysis of a GFP tagged RGS5 gene in yeast. (A) Halo assays were performed on yeast transformants harbouring plasmid p426GAL1-ATG-GFP (GFP) or p426GAL1-RGS5-GFP (RGS5-GFP). Sensitivity to the growth inhibitory effects of the yeast GPCR agonist is proportional to the zone of no growth surrounding the α -factor containing filters. (B) Western blot analysis using RGS5 anti-serum with extracts prepared from yeast cells expressing RGS5 or RGS5-GFP. The locations of the RGS5 and RGS5-GFP monomers and the putative dimers (dRGS5 and dRGS5-GFP) are shown. An “*” denotes the non-specific 87 kDa band while a double asterisks (**) denotes a band that is reproducibly observed but whose origin remains unknown. Molecular weight markers in kDa are shown on the right of the blot. (C) Western blot analysis, using GFP anti-sera, of extracts prepared from yeast cells expressing GFP alone and GFP fused to the C-tails of different GPCRs (SST₅, somatostatin receptor 5, 56 residues, GenBank accession # NM_001053; β_2 AR, β_2 -adrenergic receptor 2, 90 residues, accession # BC032883; CB₁, cannabinoid receptor1, 70 residues, accession # NM_007726; and LPA₄, lysophosphatidic acid receptor 4, 57 residues, accession # NM_020028). All western blots were performed at least three times with similar results.

cross-reactive protein. In contrast, the 42kDa protein appears to be a modified form of RGS5, since it is not seen in control yeast cells that do not overexpress RGS5 (Fig. 1A). Because the 42 kDa protein corresponds to the approximate MW of two RGS5 proteins, we hypothesized that it may represent a RGS5 dimer. The putative RGS5 dimer is also detected in extracts prepared from HEK293A cells that are overexpressing RGS5 (Fig. 1B). This later result suggests that the putative dimer is not an artifact of overexpressing RGS5 in the heterologous yeast cell. Using the same affinity purified anti-sera, we previously reported that RGS5 protein could only be detected in skeletal and cardiac muscle tissue and cell lines [18]. We were unable to detect the putative RGS5 dimer in extracts prepared from these tissues or cells because of the large number of cross-reactive proteins that were recognized by the anti-serum (not shown).

Analysis of an RGS5-GFP fusion in yeast

R4 RGSs are known to interact with other proteins [4, 13]. Although the interaction of RGS5 with G_{α} proteins is likely to be transitory, the strength of its interaction with other proteins such as the chaperone 14-3-3 is not well known [13]. Since many yeast and human proteins share similar sizes and function, it remains possible that the putative RGS5 dimer that we observe actually represents a dimer that forms between RGS5 and another protein. To further investigate the possibility that we observe an RGS5 dimer, we constructed an RGS5-GFP fusion gene in order to express a GFP tagged RGS5 protein in yeast. Given that the RGS5-GFP protein has a calculated MW of 47.7 kDa, we should be able to detect a 95 kDa protein if RGS5-GFP does indeed form a dimer with itself. As a first step towards characterizing the RGS5-GFP fusion protein, we used a yeast mutant lacking

Fig. 3. Analysis of RGS5-RGS5 interaction using the yeast two hybrid system. (A) A schematic diagram depicting the fusion proteins used in this study is shown at the top. Yeast cells were transformed with different combinations of bait (BAIT) and prey (PREY) expressing plasmids as indicated. Cultures of the different transformants were serially diluted and aliquots were spotted onto nutrient agar with (+His) and without (-His) histidine. SMS1¹⁻⁷⁰ represents the N-terminal 70 residues of sphingomyelin synthase (GenBank accession no. AY509044) and WIPI49 is a two hybrid clone that was found to interact with SMS1¹⁻⁷⁰ in a two hybrid screen of a mouse cardiac cDNA library (Dualsystems Biotech AG). Growth in the absence of histidine (-His) indicates that an interaction is occurring between the bait and the prey tested. Similar results were obtained in 3 different experiments using two different groups of yeast transformants. (B) Extracts prepared from yeast cells expressing the different LexA-bait protein fusions, described in A, were analyzed by western blot using a specific LexA anti-serum.

its RGS-encoding *SST2* gene to determine if RGS5 retained RGS function when fused to GFP. *Sst2* yeast cells are hypersensitive to GPCR stimulation and are consequently hyper-responsive to the growth inhibitory effects of the GPCR agonist α -factor. We and others have shown that a number of different mammalian RGSs, including RGS5, can inhibit endogenous GPCR signalling and confer an increased resistance to α -factor when expressed in yeast [1, 4, 19, 27]. Using the halo assay, we show that cells transformed with the control plasmid p26GAL1-ATG-GFP (which expresses GFP alone) show a significant zone of no growth around the filter discs containing α -factor indicating a strong GPCR mediated response (Fig. 2A). In contrast, expression of RGS5-GFP attenuated the growth inhibitory effects of α -factor (Fig. 2A, bottom plate). These results indicate that RGS5, like a number of other RGSs, can be fused to GFP and retain its ability to function as an RGS [20, 28, 29].

Western blot analysis was then carried out using extracts prepared from yeast cells expressing RGS5 or RGS5-GFP. As seen in Figs. 1A and 2B, we observe 2 specific bands in cells expressing RGS5 using the RGS5 anti-serum. The 23 kDa band represents the RGS5 monomer while we have named the 42 kDa band dRGS5 to reflect the fact that it may be a RGS5 dimer. Both of these bands are absent in cells expressing RGS5-GFP. Instead, we observe a prominent band having a calculated MW of 50 kDa, which corresponds closely to the calculated MW of 47.7 kDa for RGS5-GFP (Fig. 2B). In

addition, a band having an estimated MW of 100 kDa is also present. The size of the band, which we have named dRGS5-GFP, has the appropriate MW to represent a RGS5-GFP dimer. These results strongly suggest that the observed 42 kDa that cross-reacts with the RGS5 anti-sera is indeed an RGS5 dimer.

GFP has been reported to be capable of self-dimerizing under some conditions [30, 31]. In order to rule out the possibility that the observed RGS5-GFP doublet is due to GFP itself, we performed a western blot analysis using protein extracts prepared from yeast transformants expressing GFP alone or GFP fused to the C-terminal regions of a number of different GPCRs (Fig. 2C). Using specific GFP anti-serum, a single band corresponding to the appropriate MWs of GFP or the GFP fusions was detected (Fig. 2C). Thus GFP does not form a dimer that is detectable by western blot analysis when expressed in yeast.

Fig. 4. Analysis of the stability of the RGS5 monomer and dimer. Identical cultures of HEK293A cells expressing hRGS5 were treated with cycloheximide (CHX, 50 μ g/ml). Samples were harvested prior to the addition of CHX (0) and after 4, 8, and 24 hours (hr). Extracts were prepared and analyzed for RGS5 expression by western blot. Similar results were obtained in two independent experiments.

RGS5-RGS5 interaction by two-hybrid analysis

Although the previous results clearly indicate that RGS5 can form a dimer, it remains that this dimer may be a methodological artifact. We therefore made use of the yeast two-hybrid system as a different approach to determine if we could detect RGS5-RGS5 interaction [24]. Since the yeast strain used has a LexA-*HIS3* fusion reporter gene, histidine prototrophy indicates that a given bait and prey are physically interacting. The spot assay was therefore used to assess growth in the absence of histidine of yeast cells transformed with different combinations of bait and prey vectors that express the different protein fusions shown in Fig. 3A. As a control, we show that all yeast transformants are capable of growth when the growth media is supplemented with histidine (Fig. 3A). As a positive control, we made use of the fact that WIPI49 has been previously shown to interact with the N-terminus of sphingomyelin synthase (unpublished observation). Yeast cells harbouring the bait plasmid expressing SMS1¹⁻⁷⁰ [26] and the WIPI49 expressing prey vector are positive for growth in the absence of histidine (Fig. 3A). In addition to these plasmids, we transformed yeast with different bait plasmids expressing the N-terminus (RGS5¹⁻⁵⁹) or the C-terminal RGS box (RGS5⁶⁰⁻¹⁸¹) of RGS5 and prey vector expressing the entire RGS5 coding sequence. Of the

different combinations tested, only the RGS5 box and RGS5 were found to interact (Fig. 3A). Given that RGS5 did not interact with SMS1¹⁻⁷⁰ or that the RGS5 box did not interact with WIPI49, the observed RGS5 Box interaction with RGS5 appears to be specific (Fig. 3A). It nevertheless remained possible that the negative results demonstrating that the N-terminus does not interact with RGS5 is due to the RGS5¹⁻⁵⁹ bait protein not accumulating in yeast cells. We therefore used western blot analysis to show that all the bait proteins used in this study, including the RGS5 N-terminal-LexA fusion accumulate in yeast cells (Fig. 3B). Taken together these results provide strong evidence that RGS5 is capable of self-interacting.

RGS5 dimer is more stable than the RGS5 monomer

The levels of many of the R4 RGSs, including RGS5, are regulated in part by post-translational mechanisms [4, 6, 32-34]. We therefore wanted to examine the possibility that the RGS5 dimer may represent a sub-population of RGS5 proteins that are differentially regulated than the monomer. To examine the stability of RGS5, HEK293A transfected with pcDNA-hRGS5 were treated with cycloheximide and samples were harvested at intervals for western blot analysis using RGS5 specific anti-sera. Upon blocking protein synthesis with cycloheximide, the levels of RGS5 monomer were found to decrease with a half life of 20 hours (Fig. 4). In contrast, the RGS5 dimer remained stable with 100% of the protein still present 24 hours after the addition of cycloheximide (Fig. 4). This demonstrates that RGS5 dimer is differentially regulated than the monomer.

Discussion

Members of the R4 RGS sub-family are best known as proteins that can serve as GAPs for subsets of different heterotrimeric G α proteins [4-8, 35]. Our understanding of their involvement in cellular processes such as their ability to function as effector antagonists as grown alongside our understanding of the diversity of proteins that interact with the R4 RGSs [9,13]. The list has grown to include chaperones, GPCRs, effectors and adapter proteins. Here we add to this list by providing the first report that describes the dimerization of a member of the R4 RGS sub-family. RGS5 dimerization was first detected as a protein having the MW of 2 RGS5 proteins by western blot analysis in extracts prepared from both yeast and HEK 293 cells overexpressing RGS5 (Fig. 1).

Detection of a RGS5-GFP dimer in the extracts prepared from yeast cells expressing a functional RGS5 cDNA tagged with GFP served to confirm that RGS5 is capable of dimerizing (Fig. 2). The ability of the RGS5 and RGS5-GFP dimers to remain intact after SDS-PAGE suggests that the observed RGS5-RGS5 dimer is resistant to detergent and reducing agents that are used to prepare the samples. Although this may reflect covalent attachment, non-covalent interaction may also lead to such strongly attached dimers. The situation is reminiscent of other non-covalently attached dimers, such as GPCRs, that have been shown to remain attached under such stringent conditions [36]. The nature of the RGS5-RGS5 dimer remains at present unknown.

Yeast two hybrid analysis was used to confirm that that RGS5 was capable of self-interacting (Fig. 3). Our results suggest that the RGS5 interacting region is present within the RGS box. These results are somewhat surprising since the RGS box is thought to be the site of interaction with G_{α} -proteins while the N-terminal region has been shown more versatility since it can interact with a number of different proteins including GPCRs, effectors and some adapter proteins [11, 13]. The involvement of the RGS box suggests that RGS5-RGS5 interaction may have a regulatory role. For example a number of R4 RGSs interact with 14-3-3 and this interaction may serve to inhibit RGS GAP activity [13]. It would be interesting to determine if RGS5 dimerization serves a role in modulating the GAP activity of RGS5. We previously demonstrated that RGS5 protein is abundantly expressed in cardiac and skeletal muscle [18]. Basal levels of RGSs are known to limit GPCR responsiveness while decreases in the basal levels of RGSs increase GPCR responsiveness [4, 6]. Alterations in the activity of RGS5 by dimerization may therefore serve to regulate a subset of the large number of GPCRs that are known to be expressed in cardiac and skeletal muscle [37, 38].

Analysis of the stability of RGS5 ectopically expressed in HEK293 cells suggested that the RGS5 monomer has a shorter half-life than the dimer (Fig. 4). These results are consistent with previously published reports showing that RGS5 has a relatively short half-life [32-34]. The N-end rule ubiquitin dependant pathway has been shown to be involved in degrading RGS5 [33, 34]. Thus dimerization of RGS5 appears to prevent the rapid degradation of RGS5 by as of yet unknown process that

may simply involve the masking of the N-terminal region of RGS5. RGS5 levels, as observed for a number of other RGSs, are known to be increased in response to GPCR stimulation [4, 8, 18]. We nevertheless find no difference in the levels of the RGS5 monomer in HEK cells overexpressing RGS5 that are stimulated with GPCR agonists (not shown). Although the differential stability of the monomer versus the dimer is consistent with our results, it nevertheless remains possible that the RGS5 monomer is not lost but instead it could be converted to the dimeric form. Experiments using proteasome inhibitors are underway in order to further examine these possibilities.

Although the possible significance of RGS dimerization is not known, it is worthwhile mentioning that the significance of GPCR dimerization was not well understood when it was first reported [36]. Nevertheless, today it is quite clear that GPCR-GPCR interaction is a widespread phenomenon that plays critical roles in multiple aspects of the biology of these receptors [17]. The implications for RGS5 function in regulating GPCR signalling as well as the possible dimerization of other R4 RGSs will be questions of great interest. In this context, it is interesting to note that purified RGS4 appears to form a dimer in vitro [33]. Although RGS4 and RGS5 along with RGS8 and RGS16 may be a subfamily R4 RGSs [19], these results suggest that RGS dimerization may be a common phenomenon.

Abbreviations

GFP (Green fluorescent protein); GPCR (G-protein Coupled Receptor); RGS (Regulator of G-protein Signalling); SMS (Sphingomyelin synthase).

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