Comparative Analysis of the Regions Binding βγ-Subunits in Gα and PH Domains

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Some of the pleckstrin homology (PH) domains are shown to bind βγ-subunits of a G-protein. In this paper we present a detailed comparison of sequences and structural features of βγ-binding regions in PH domains and α-subunits (Gα) of known structure. Our comparison involves switch II region in Gα and a C-terminal region of PH domain structures and we suggest a similarity in these regions. By comparing the active and inactive forms of Gα structures we predict that some of the bulky hydrophobic residues in switch II region might interact with βγ-subunits. Subsequent to completion of this work we find that this prediction is absolutely consistent with recently reported crystal structures of heterotrimeric G-proteins. We discuss the feasibility of common principles in the recognition Gα and PH domains by βγ-subunits. © 1996 Academic Press, Inc.

The heterotrimeric guanidine nucleotide binding proteins (G-proteins) recognise activated receptors through their α-subunits (Gα) leading to the replacement of guanosine diphosphate (GDP) by triphosphate (GTP) and dissociation from the βγ-subunits. When the GTP is hydrolysed to GDP, the Gα reassociates with βγ-subunit, and the cycle continues enabling transmission of signals from a receptor to an effector. Gα is made up of two structural domains: a GTPase domain consisting of a six-stranded β-sheet surrounded by six helices and the other domain comprises of a long α-helix surrounded by five other α-helices (1–4). The suggested βγ-binding sites of Gα are the N-terminal region of about 23 residues and the "switch II" region in the α/β domain that has different structure depending on if Gα is bound to GTP or GDP.

Some pleckstrin homology (PH) domains are shown to bind βγ-subunits. They include phospholipase Cβ (PLCβ), β-adrenergic receptor kinase (βARK) and Bruton tyrosine kinase (BTK) (for recent reviews see, 5–9). The tertiary structure of a PH domain consists of two orthogonal β-sheets and an α-helix at the C-terminus (10–18). The βγ-binding region corresponds to the terminal α-helix and probably further residues towards the C-terminus (19–22) which are not present in the known structures.

Here we present a detailed comparison of the sequences and structures of the switch II region and C-terminal of PH domains and highlight the similarities. We also discuss the feasibility of common principles in the recognition Gα and PH domains by βγ-subunits.

MATERIALS AND METHODS

The different structures used in our analysis, complexes of Gα-GDP and Gα-GTP and the structures of PH domains from dynamin and pleckstrin, are from Brookhaven Protein Data bank (23). The structural similarity in the βγ-binding regions of Gα and PH domains are detected by visual inspection of the structures using the interactive graphics software *SETOR* (24). In order to compare the structural features at the residue positions using the program *JOY* (25) we calculated the secondary structure, solvent accessibility and hydrogen bonding pattern at every residue position. The secondary structure calculation was performed using the program *SSTRUC* (Smith and Thornton, Unpublished) which employs the *DSSP*

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algorithm (26). The solvent accessibility at the various residues were computed using the program *PSA* (Sali and Overington, Unpublished) and the hydrogen bonds were detected using *HBOND* (Overington, Unpublished).

The search for sequences of $\beta\gamma$-subunit binding regions of PH domains and Gα was performed in the *SWISSPROT* database employing the Smith-Waterman algorithm which is effective for detecting similarities in a local region of a much larger sequence.

BACKGROUND, RESULTS AND DISCUSSION

The N-terminal segment of Gα is a highly hydrophilic segment, not seen in most of the crystal structures but thought to be a disordered helix pointing away from the globular structure of α-subunits (1–4). Most small G-proteins such as *ras* p21 lack this N-terminal segment and do not bind to $\beta\gamma$-subunits. However, ADP ribosylation factor 1 (ARF1), a small G-protein which binds $\beta\gamma$-subunits, does contain an equivalent N-terminal segment (27). Although ARF1 shares some features with variants of Gα, the N-terminal segment of ARF1 shows no detectable aminoacid sequence similarity with the equivalent region of Gα variants (27). In the crystal structure of ARF1 the N-terminal helix is visible and is seen to fold back making close contacts with the GDP binding domain (28,29). Recently the N-terminal segment of a Gα structure has been shown to form a microdomain on GDP hydrolysis and interacts with the neighbouring α-subunit in the crystal lattice (30). Hence the role of this N-terminal segment in $\beta\gamma$-binding may be slightly different in different systems.

The other region implicated in binding $\beta\gamma$-subunits includes a helical segment between 201 and 210 in the switch II region of the α/β domain of Gαt (6,31). This region has significantly different structures in the GTP and GDP bound states of a Gα (3). Interestingly a cysteine in the α2 helix in the switch II region can be cross-linked to the C-terminus of the β-subunit (32) giving evidence for the involvement of this region in binding $\beta\gamma$-subunits.

This helical region is also an effector binding site in certain variants of Gα such as α_o but simultaneous binding of effector and $\beta\gamma$-subunits is unlikely. In transducin Tα a tryptophan (Trp 207) in this helix is the fluorescence sensor of the G-protein activation switch in the activation of an effector (PDE) (33). It is further shown that the mutation of this Trp to Phe hampers the activation of PDE but does not affect the binding to receptor or $\beta\gamma$-subunits (33). This Trp is situated two residues before Cys210 which can be cross linked with the $\beta\gamma$-subunits (32) suggesting that Trp (Phe in the mutant) is nonetheless proximal to the $\beta\gamma$-subunits in the complex. This bulky hydrophobic nature at this residue is conserved in ARF1, although it is not conserved in other small G-proteins and is substituted by very different residues such as Arg in p21 *ras* which do not bind to $\beta\gamma$-subunits.

Recently $\beta\gamma$-subunits have been shown to bind to the PH domain of βARK, albeit less strongly than the construct with additional 33 residues following the βARK PH domain (22). The only residue that is conserved in all PH domain sequences is a Trp in the C-terminal helix region (12). This Trp is buried in all known PH domain structures and has not been considered a likely candidate for binding $\beta\gamma$-subunits. While the work of Mahadevan *et al* (22) showed that a construct containing residues up to the helical region is sufficient for binding to $\beta\gamma$-subunits the importance of the tryptophan in the helix in binding $\beta\gamma$-subunits is disputed. On the one hand it is reported that reconstitution assays of wild type PLC-β3 and its three mutants with the absolutely conserved Trp replaced by either Phe, Ala and Gly show no significant alteration of affinity for $\beta\gamma$-subunits (34) and presumably no major alteration in the tertiary structure. On the other hand, competitive binding experiments of the wild type BTK and Trp $\rightarrow$ Gly mutant show that the presence of a Trp is important for $\beta\gamma$-binding (20). This is supported by the fact that, whereas a fusion protein of βARK inhibits $\beta\gamma$-subunit/phospholipid (PIP2)-dependent βARK activity, the Trp $\rightarrow$ Ala mutant has no inhibitory activity (35). Replacement of Trp may cause a significant disruption to the overall structure and that of $\beta\gamma$-binding region. While integrity of the fold of the Trp mutants is unknown the inactivity of these mutants could alternatively be due to direct involvement of Trp in the wild

type in binding $\beta\gamma$-subunits. More recently it was shown that a mutant of βARK with an insertion of Ala following the Trp did not bind $\beta\gamma$-subunits which further emphasises that this region is sensitive to binding of $\beta\gamma$-subunits (36).

The three-dimensional structures of the local regions comprising putative $\beta\gamma$-binding helices in PH domain and GTP-bound α-subunit are shown in Fig. 1a and b. The region of gross structural similarity extends up to the two β-strand regions before the helix, although the overall folds of the two proteins are different. There is a subtle difference in the way the N-terminus of the first β-strand is packed with the C-terminal of the second β-strand. These terminal regions of β-strands are flipped in their positions in the two structures. However the interactions between the termini of the two β-strands are variable even between the Gα structures bound to different ligands (GTP or GDP). In both cases, the conserved Trp is buried in the interface between the helix and the β-hairpin. The helical segment in the GTP bound form of the α-subunit is α-helical at the N-terminus but becomes a 3_{10} helix towards the C-terminus. The equivalent region of the PH domain structures is fully α-helical.

The amino-acid sequences of the putative $\beta\gamma$-binding regions of PH domains and Gαt are similar (Fig. 2A). Structural environments at the individual residues are also indicated for proteins of known structure. The only absolutely conserved residue among PH domains, Trp108 aligns with conserved Trp207 of Gαt, a feature independently noticed by other groups (7,8,27). Considering two sequences at a time, the sequence identity among these regions of PH domains varies between 9% and 33%. Interestingly the most similar of the corresponding regions of Gα and PH domains have 27% (with BTK) and 21% (with dynamin) sequence identities. Fig. 2B shows the alignment of the α-subunit with PLCβ and sequences similar to PH domains such as PI3 kinase γ (37) and *Drosophila melanogaster* G-protein coupled receptor kinase (21); all bind to $\beta\gamma$-subunits. The sequences are similar with the best sequence identity involving α-subunit are 23% (with PI3Kγ) and 22% (with PLCβ).

The structure of the GDP bound α-subunit, which forms a heterotrimer with $\beta\gamma$-subunits, shows that this helical segment and the preceding β-strands undergo considerable structural changes (3). The first of the two β-strands becomes shorter at the beginning of the strand resulting in a slightly different packing with the C-termini of the following β-strand. The C-terminus of the helix

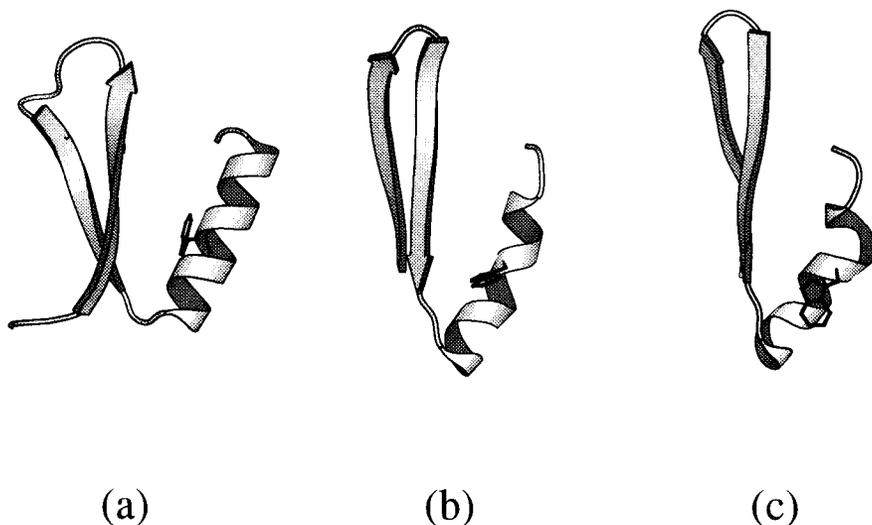(a)   (b)   (c)

FIG. 1. Ribbon representation of the course of mainchain in the putative $\beta\gamma$-binding regions of dynamin PH domain (left) (13), active form (GTP bound) of transducin (middle) (1), and inactive form (GDP bound) of transducin (right) (3). The conserved Trp is also shown in all these structures. This figure was generated using *MOLSCRIPT* (43).

becomes fully 3_{10} helical but a few residues shorter in length (Fig. 1c). This change exposes two of the otherwise buried hydrophobic residues Trp and Phe, indicating that they may contribute to some extent in the $\beta\gamma$-binding. Fig. 2A shows that the equivalents of these two residues in PH domains are conserved or conservatively varied. We do not have an experimental structure of a PH domain comparable to the GDP bound form of Gαt. The location and detailed conformations of C-terminal helix of the PH domains are variable, possibly due to flexibility of this helix. Although superposition of two independent determinations of the crystal structure of dynamin PH domain (13,14) shows that the structures are extremely similar including the sidechain positions in the helical region, superposition of a representative NMR structure (12) and the crystal structures of dynamin PH domain shows that there are small differences in conformations at the end of the C-terminal helix such that the positions of the sidechains of Trp108 and Phe112 are slightly
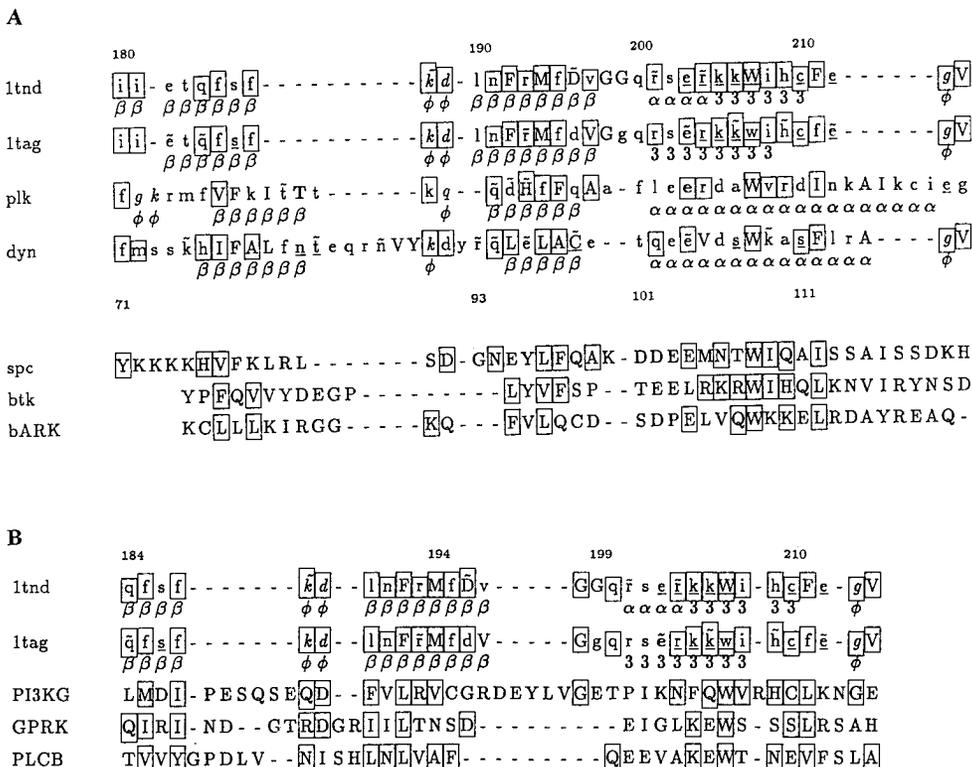

FIG. 2. (A) Structure-based sequence alignment of putative $\beta\gamma$-binding regions of transducin (1tnd and 1tag), dynamin PH domain (dyn), and a representative NMR structure of pleckstrin (plk). Structural environments at the individual residue positions are indicated by *JOY* notation (25) and the secondary structural regions (β - β-strand; α - α-helical; 3 - 3_{10}-helical). Key to *JOY* notation: solvent inaccessible, uppercase (X); solvent accessible, lowercase (x); positive ϕ, italic (*x*); *cis* peptide, breve (); hydrogen bond to other sidechain, tilde (); hydrogen bond to mainchain amide, bold (**x**); hydrogen bond to mainchain carbonyl, underline (x); disulphide bond, cedilla (). 1tnd (1) and 1tag (3) represent crystal structures of GTP bound (active) and GDP bound (inactive) forms, respectively. dyn corresponds to the crystal structure by Timm *et al.* (13) and plk corresponds to the solution structure of pleckstrin determined by Yoon *et al.* (10). Most of the coordinate sets used in our analysis were obtained from Brookhaven Protein Data Bank (23). The numbering at the top and bottom corresponds to transducin and dynamin PH domains, respectively. Sequences at $\beta\gamma$-binding regions of other PH domain of known 3-D structure (spectrin - spc) and those which are shown to bind $\beta\gamma$-subunits (Bruton tyrosine kinase, btk, and β-adrenergic receptor kinase, bARK) are also aligned. (B) Sequence alignment of putative $\beta\gamma$-binding regions of transducin and some analogues of PH domain that bind $\beta\gamma$-subunits. Structural environments at the residue positions in the two crystal forms of transducin are also indicated. PI3K, human phosphatidylinositol 3-kinase γ; GPRK, *Drosophila melanogaster* G-protein coupled receptor; PLCB, phospholipase Cβ. Residue positions that are conserved or conservatively replaced between transducin and PH domains/PH domain analogues are shown boxed in (A) and (B).

reoriented although these sidechains are in almost identical positions in the two crystal structures. Also the superposition of a representative solution structure of pleckstrin (10) and the crystal structure of dynamin PH domain (13) indicate that while most of the β-strands are well overlayed, the helical segments are not very well superposed. Clear high displacements in the overlayed Cα atoms in the helical regions can be seen. The C-terminal helix of dynamin PH domain is shorter by 4 residues compared to pleckstrin (Fig. 2A) and it further contributes to the structural differences.

We have searched the database of known three-dimensional structures for segments which share sequence and structural similarity with the C-terminal $\beta\beta\alpha$ region of PH domains and the equivalent region of Gαt. Although smaller segments similar to either helical or β-strand regions have been found, no segment with sequence and structural similarity with the full length $\beta\beta\alpha$ regions of either PH domains or Gαt have been identified. We have also searched in protein sequence databases for proteins containing segments with sequence similarity to the C-terminal region of PH domains. Apart from identifying variants of PH domains themselves, variants of Gα could be picked-up. The hits included proteins known to have GTPase activity (such as 35.5 kDa protein in chromosome III) and others involved else where in signal transduction (Data not shown). A search for the local region on Gαt resulted in recognition of various homologues of Gα followed by variants of ARF. Interestingly the *vav* oncogene product and dynein heavy chain are also identified by a search for the β-strand - β-strand - helix region of PH domains. It remains to be seen for a number of such proteins if they bind $\beta\gamma$-subunits and/or possess GTPase activity.

Subsequent to completion of this analysis the crystal structures of heterotrimeric G-proteins (38,39) and the structure $\beta\gamma$-subunits not bound to Gα (40) have appeared. The $\beta\gamma$-binding site in Gα comprises of two regions. While the N-terminal segment is involved in less extensive contacts with $\beta\gamma$-subunits, most of the binding takes place through the residues in the $\beta\beta\alpha$ motif of the switch II region. This is completely in agreement with our prediction which was mentioned in our preliminary communication (41). The hydrophobic residues Trp207 and Phe211 in the switch II helix that is exposed in the GDP bound form are interacting with hydrophobic partners in the β-subunit further validating our prediction. It is intriguing to note, from Fig. 2, that hydrophobic nature at these residues are conserved among PH domains that are shown to bind $\beta\gamma$-subunits.

In conclusion the local regions of α-subunits and PH domains potentially involved in binding to $\beta\gamma$-subunits share some similarity in aminoacid sequence and structure. The dissimilarities in the $\beta\gamma$-recognition by Gα and PH domain may be contributed by the involvement of N-terminal segment of Gα and extended C-terminal of PH domain in binding. However, the suggested analogy between Gα and PH domain might imply some similarities in the recognition of $\beta\gamma$-subunits. A significant structural alteration at the C-terminal helix of the PH domain may take place on binding to $\beta\gamma$-subunits although the details of structural change can be different from that in a Gα. Much of the potential analogies remain to be explored by structure determinations of the complexes, fluorescence and other spectroscopic analyses. While the conserved Trp of PH domains may be important for $\beta\gamma$-binding it appears that either of Trp and Phe can perform the binding role. For example the recently determined crystal structure of phosphotyrosine binding domain of Shc is unexpectedly has the same fold as PH domains (42) with the conserved Trp of PH domains replaced by a Phe. It is however suggested to bind $\beta\gamma$-subunits (42).

ACKNOWLEDGMENT

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