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Hypothesis

Do G protein subunits associate via a three-stranded coiled coil?

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We used a computer-based prediction algorithm to identify probable coiled-coil segments at the N-termini of G protein α, β and γ subunits. This result indicates that G protein trimers may form via a three-stranded coiled coil. Previous biochemical results had shown that the N-termini of α and β are involved in subunit interactions. Here we present a structural model for the N-terminal domain of $\beta\gamma$ and a hypothesis for the reversible association of α to $\beta\gamma$.

Coiled coil; G protein trimerization, G protein structure, Transducin $\beta\gamma$ dimer

1. INTRODUCTION

Heterotrimeric guanine nucleotide-binding proteins (G proteins) (reviewed in [1–3]) mediate responses to stimuli detected by cell surface receptors with seven transmembrane helices. The nucleotide-binding site is located in the α subunit, which associates with $\beta\gamma$ in its GDP-bound form and dissociates when a stimulated receptor induces the exchange of GDP for GTP. In the active, dissociated form, both α and $\beta\gamma$ interact with effector proteins such as ion channels or enzymes that generate second messengers [4–6]. The G protein deactivates when the intrinsic GTPase activity of the α subunit causes it to hydrolyze the bound nucleotide and reassociate with $\beta\gamma$.

The structural basis for interactions between α, β, and γ has not been established, but biochemical results suggest that the N-termini of α and β are involved in trimerization. In the transducin $\beta\gamma$ dimer, Cys^{36} of the γ subunit is readily crosslinked to Cys^{25} of the β subunit [7], indicating that γ interacts with the N-terminal domain of β. In several α subunits (α_t, α_o, α_i), partial proteolysis results in the loss of an N-terminal peptide of approximately 20 residues; this renders α unable to associate with $\beta\gamma$ but does not affect its ability to interact with guanine nucleotides [8,9]. Association with $\beta\gamma$ protects the α N-terminus from cleavage [10].

We developed an algorithm to predict coiled-coil structures from protein sequences [11]. This method correctly predicted the coiled-coil domains in GCN4, seryl-tRNA synthase and apolipoprotein E before these structures were determined by X-ray crystallography [12–14]. A search of GenBank revealed many additional proteins with probable coiled-coil domains, including heterotrimeric G proteins [11]. In these, the predicted coiled-coil segments are at the N-termini of the subunits (Fig. 1). In conjunction with previous biochemical results, this finding indicates that trimerization depends, at least in part, on formation of a three-stranded coiled-coil structure.

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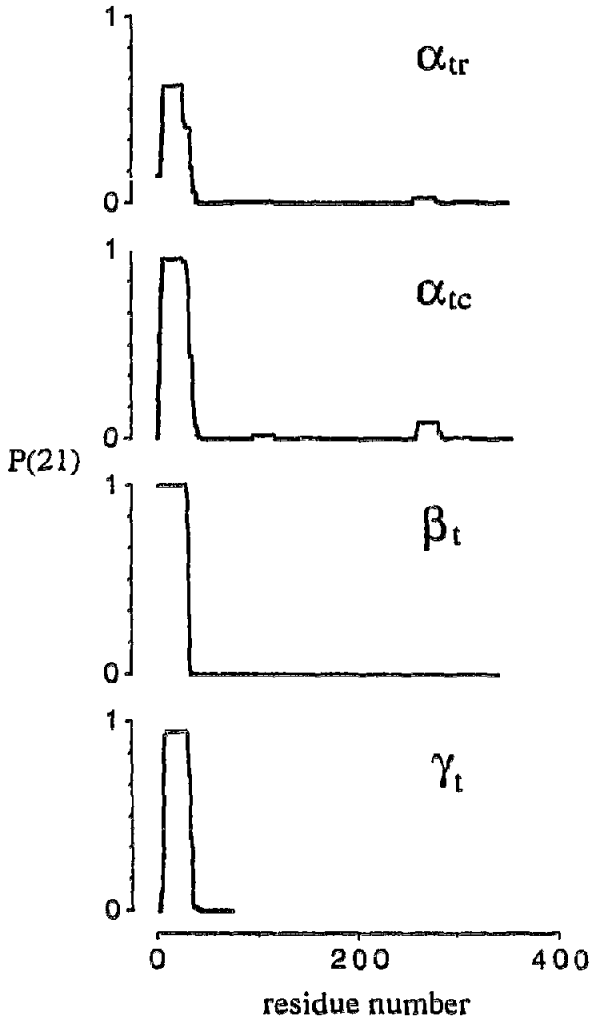
2. COILED-COIL SEGMENTS IN α, β AND γ SUBUNITS

The highest coiled-coil forming probabilities, in excess of 99.9%, are obtained by the N-terminal domain of β subunits (Met^1–Ser^{31} in transducin β). The yeast mating type β subunit Ste4, which shows considerable sequence divergence from the other β subunits and contains 35 additional residues at its N-terminus, is predicted to contain a coiled-coil of approximately six heptads (Gln^{23}–Leu^{65}); the last four of these are homologous to the heptads found in the other β subunits. The 35 N-terminal residues of Ste4, which include the first two heptads of the predicted coiled-coil, can be removed without loss of function [15] indicating that, as in the other β subunits, 4 heptads are sufficient to form a complex with α and γ.

Coiled-coil forming probabilities for γ subunits are lower than for β subunits and generally do not exceed 50%. Nevertheless, the probability of 95% obtained for γ_1 (Fig. 1) clearly highlights the coiled-coil forming potential of γ. In all γ subunits, the first two heptads, corresponding to Lys^{12}–Gly^{25} in γ_1, obtain the highest



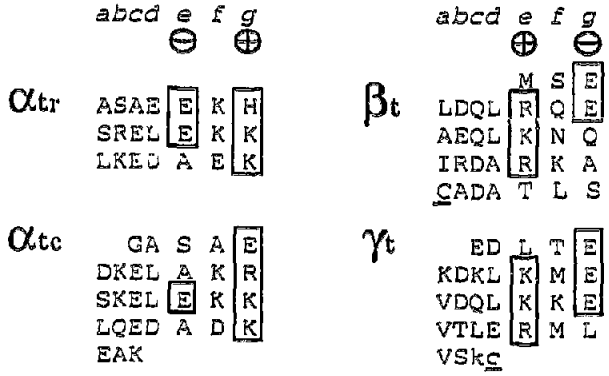


Fig. 1. Coiled-coil segments in transducin α (rod and cone), β, and γ. The graphs show the coiled-coil forming probabilities obtained in 21 residue scans. The coiled-coil prediction algorithm used [11] is available as a VAX Pascal program from the authors. At the bottom are shown the predicted heptads. Two residues not part of the heptad repeat are included in lowercase letters in γ_t to show Cys^{36}, which can be crosslinked to Cys^{25} in β_t (both cysteines are underlined). Charged residues in positions *e* and *g* are boxed.

scores and the low probabilities are mainly due to the third heptad that tends to diverge significantly from the coiled-coil consensus. As in β subunits, the yeast mating type γ subunit, Ste18, is the most divergent of all sequenced γ subunits and contains a coiled-coil of 5–6 heptads (Gln^{19}–Ala^{56}) with a discontinuity after Lys^{28}.

The α subunits scores show the greatest variability: only about half of the analyzed α subunits obtain prob-

family	subunit	*defgabcdefgabcdefgabcdefgabc*
G_s	G_s	mgclgnsktEDQRNEEKAQREANKKIEKQLQKDKQvyratββββββ
G_i	G_{i1}	mgctvsaedkaaaersknidknlredgekaareββββββ
	G_o	mgcTLSAEERAALERSKAIEKNLKEdgisaakdββββββ
	T_r	mgagASAEEKHSRELEKKLKEDAEKdartββββββ
	T_c	mgsGASAEDKELAKRSKELEKKLQEDADKEAKtββββββ
	G_z	mgcrqsseekeaarrsrridrhlrsesqrqrreββββββ
G_q	G_q	mtlesimacCLSEEAKEARRINDEIERQVRRDKRdarreββββββ
	G_{16}	marsltwrccpwcltedekaaarvdqeihriilleqkkqdrgeββββββ
G_{12}	G_{12}	msgvvrtlsrcllpaeagarerragaardaereaarrsrdiiaillarerravrriββββββ
	G_{13}	madflpsrsvlsvcfpgcvltngeaeqqrkskeiikcllsrektyvkrlββββββ

Fig. 2. Coiled-coil segments in mammalian G protein α subunits. The probable first β strand in the guanine nucleotide-binding domain [16,17] is shown as *ββββββ*. Predicted coiled-coil segments are shown bold in uppercase letters; P(21) probabilities were: G_s 89%, G_o 79%, T_r 62%, T_c 96%, G_q 83%. Coiled-coil segments were also detected with significant probabilities in non-mammalian G α subunits from *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Dyctiostelium discoideum* and *Saccharomyces cerevisiae*. Two residues that are highly conserved in all G α subunits are boxed. Arrowheads mark the position of proteolytic cleavage in G_s and T_r [8].

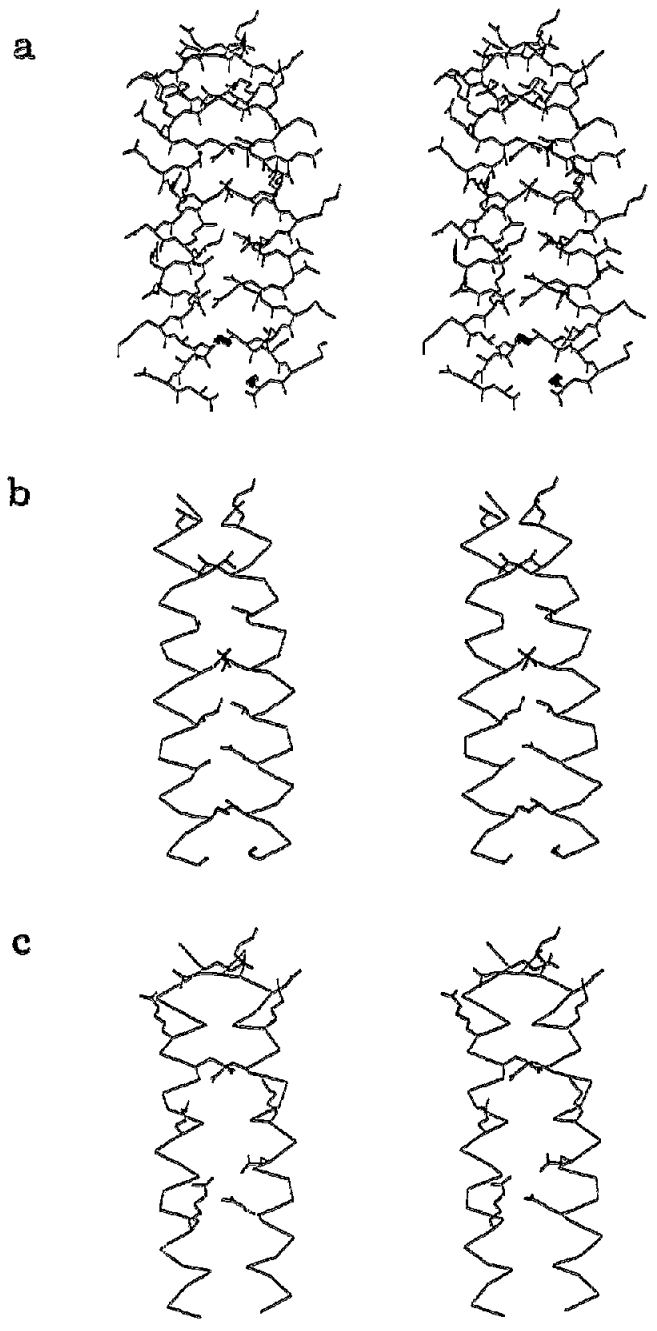


Fig. 3. Stereo-views of a model for the predicted coiled-coil in transducin $\beta\gamma$, showing residues 3–28 of β_1 and 11–36 of γ_1. In (a) the complete model is shown with Cys^{25} of β_1 and Cys^{36} of γ_1 bold. The residues forming the hydrophobic core (positions *a* and *d*) are shown in (b) and residues forming salt bridges at the interface of the dimer (β_3–γ_{12}–γ_{13}–γ_{16}; β_8–γ_{11}; β_{10}–γ_{23}; β_{15}–γ_{18}; β_{22}–γ_{25}–γ_{29}) are shown in (c). The model was built in INSIGHT/DISCOVER (Biosym, San Diego, CA) using residues 1–26 of the GCN4 leucine zipper domain [12] as a coiled-coil template and energy-minimized with the CVFF force field, explicit solvent, and a 10 Å cutoff for nonbonded interactions. PDB format coordinates are available from the authors.

abilities higher than 50% and the probabilities vary widely between members of the same α subunit class (Fig. 2). Nevertheless, although α N-terminal sequences are highly divergent, they share a conserved underlying heptad periodicity: all α subunits with discernible coiled-coil segments were assigned the same heptad repeat frame, except yeast Gpa1 (Fig. 2). Also, the structural assignment of these sequences to coiled-coils is supported by their α-helical character in secondary structure analysis [16,17]. Finally, experimental evidence strongly supports a role for these sequences in trimerization.

3. A MODEL FOR THE $\beta\gamma$ N-TERMINAL DOMAIN

We used the sequences of transducin β and γ to build a structural model for the N-terminal domain of the $\beta\gamma$ dimer (Fig. 3). The proposed interface shows surprising similarity to the interface of the GCN4 leucine zipper domain [12], including a preference for β-branched residues in position α and γ-branched residues in position *d* (Fig. 3b), and a large number of interchain ionic interactions between residues in position *g* of one heptad and *e* of the next heptad (Fig. 3c). As predicted from theoretical considerations [18] and confirmed in the structure of GCN4, no ionic interactions are observed between residues in *e* and *g* of the same heptad. Unlike GCN4, the $\beta\gamma$ dimer contains two charged residues in the hydrophobic core which are contributed by the γ subunit (Lys^{12} in *a* and Glu^{29} in *d*). These bend out of the core and are stabilized by interactions with Glu^3 and Arg^{22} of the β subunit. Similar interactions may stabilize the two lysine residues contributed by Fos to the hydrophobic core of the Fos-Jun leucine zipper [19].

We tested the quality of our structural model with the method of Lüthy et al. [20] and obtained an average 3D-1D score per residue of 0.32; this score is comparable to scores obtained by NMR structures and is equal to the best score for a computationally determined structure. Our model is further supported by a cross-linking experiment, in which Bubis and Khorana [7] used a chemical oxidizer, Cu-1,10-phenanthroline, to induce a disulfide bond between Cys^{25} of β_1 and Cys^{36} of γ_1, demonstrating the close proximity of these residues in the $\beta\gamma$ dimer. In our model, the sulfur atoms of the two cysteines are only 5.3 Å apart, one in position *a* and the other in position *d* of the hydrophobic core (Fig. 3b).

It appears unlikely that the exceptional stability of the $\beta\gamma$ dimer [21] could be provided entirely by a coiled coil of only 4 heptads. Recently, Pronin and Gautam [22] showed that γ_1 does not bind to β_2, but binds to a chimeric β_2–β_1 protein containing only the 59 N-terminal residues of β_2. This experiment indicates that other regions in β and γ interact and contribute to the overall stability and specificity of the dimer.

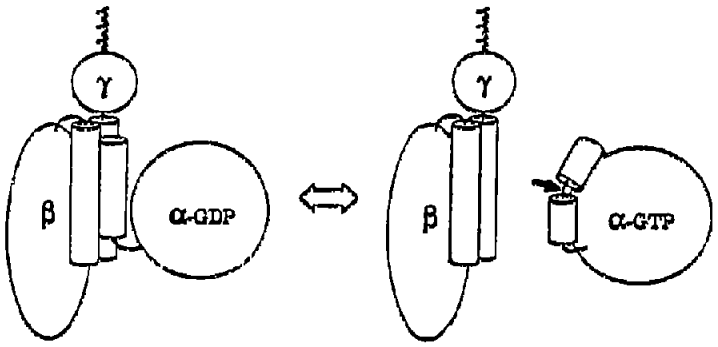


Fig. 4. Schematic model for the reversible association of α to $\beta\gamma$. The arrow indicates the site of proteolytic cleavage in the monomeric α subunit that is protected in the trimer. A polyisoprenoid lipid attached to a C-terminal cysteine in the γ-subunit is also indicated. This group is required for the association of $\beta\gamma$ with membranes [26].

Although coiled-coil segments were predicted in α_{ic} and α_{tr} with significant probabilities, an α subunit was not included in the model because it is unclear to which side of the $\beta\gamma$ dimer it binds and whether the interaction is parallel or antiparallel. Although a parallel arrangement would be canonical, the distribution of charges in positions *e* and *g* indicates that the association of α to $\beta\gamma$ is antiparallel (Fig. 1). An analogous heterotrimeric structure with two parallel strands and one antiparallel strand has been proposed for the tropomyosin–troponin T complex [23].

4. THE REVERSIBLE ASSOCIATION OF α TO $\beta\gamma$

The relatively low coiled-coil probabilities for the α subunit N-termini may reflect their potential to assume two different conformations, one of which is not a coiled-coil structure: while β and γ form a permanent dimer and are insoluble when not coexpressed [24], presumably because of incorrect packing of the undimerized coiled-coil helices, α subunits are stable both in heterotrimeric and monomeric form and appear to undergo a stabilizing conformational change when they dissociate from $\beta\gamma$. This conformational change exposes a site in their N-terminal domain to proteolytic cleavage; the site is located in the most strongly conserved region of the α N-termini, between two invariant aliphatic residues (Fig. 2). This site may form a reversible discontinuity that is induced by the exchange of GDP for GTP, facilitating dissociation from $\beta\gamma$ and permitting a compact packing for the α N-terminal domain after dissociation (Fig. 4).

5. CONCLUSION

Recent research has shown that the short coiled-coil domains found C-terminal to the DNA-binding domains in leucine-zipper transcriptional activators are essential for the formation of a large number of heterodimeric combinations with different promoter specificities [25]. Heterotrimeric G proteins may use the same mechanism to generate a spectrum of regulatory specificities. Coiled-coils have a small repertoire of periodically recurring interactions and are therefore particularly well suited to provide the relatively loose specificity required for such combinatorial systems of interacting proteins.

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