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Structural mimicry of DH domains by Arfaptin suggests a model for the recognition of Rac–GDP by its guanine nucleotide exchange factors

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Abstract Small G proteins cycle between an inactive form bound to GDP, and an active form bound to GTP. The two forms have different conformations and interact specifically with different partners, hence, the ability of G proteins to function as molecular switches. This view has been challenged by recent structural and biochemical studies of the Arfaptin/Por protein, which interacts equally well with the GDP- and GTP-bound forms of the G protein Rac. Here it is shown that the dimeric helical domain of Arfaptin superimposes with a monomeric helical domain from the Dbl homology domain of Tiam, a guanine nucleotide exchange factor (GEF) for Rac, in their respective complexes with Rac. This unexpected structural mimicry suggests that the Rac–GDP–Arfaptin complex resembles the low-affinity Rac–GDP–GEF complex that initiates the exchange reaction. This provides a model for the exchange mechanism where DH domains first dock onto Rac–GDP at the switch 2 before they undergo domain closure to catalyze GDP dissociation.

Key words: G proteins; Guanine nucleotide exchange factor; Structural mimicry; Effector; Rho, Rac, cdc42; Dbl homology domain

1. Introduction

Small GTP-binding proteins (referred to as G proteins hereafter) form a superfamily of related proteins that alternate between an inactive, GDP-bound form and an active, GTP-bound form. A hallmark of G proteins is their conformational plasticity in response to the nature of the bound nucleotide and protein partner (reviewed in [1]). In particular, GDP- and GTP-bound G proteins have different conformations at two regions, called the switch 1 and 2, which specify their recognition by distinct protein partners. The GDP–GTP cycle is highly regulated by guanine nucleotide exchange factors (GEFs), which stimulate the dissociation of the tightly bound GDP, and GTPase activating proteins (GAPs) that terminate the cycle by stimulating the intrinsically inefficient hydrolysis of GTP (reviewed in [2]). Upstream signals detected by the GEFs are thereby relayed to effector proteins that bind to GTP-bound G proteins much more tightly than to their inactive form (reviewed in [2]), hence, the ability of G proteins to function as universal cellular switches in major cellular processes such as signal transduction, morphology and traffic (reviewed in [3]).

Although G proteins are evolutionary highly related in sequence and three-dimensional structures, it has come as a surprise that each subfamily of G proteins has regulators and effectors that are unrelated in sequence, structure and interactions to those of other subfamilies and are thus unlikely to share a common ancestor. The only exception so far has been a remote structural relationship between the GAPs for Rho and Ras [4,5]. Furthermore, the various partners of an individual G protein have themselves unrelated folds. One of the best studied G proteins in this regard is Rac, a G protein that coordinates actin cytoskeleton organization at the plasma membrane to various aspects of cellular regulation [3]. Structures of Rac1 have been determined in a complex with GDP and the negative regulator GDI [6], in a nucleotide-free complex with the Dbl homology and pleckstrin homology (DH–PH) domains of its GEF Tiam [7], and bound to GTP analogues either alone [8] or in complex with a bacterial GAP [9,29] and with two unrelated effectors, Arfaptin/Por [10] and the p67 component of the NADPH–oxidase complex [11].

Here, an unreported structural relationship between Tiam and Arfaptin/Por, respectively a GEF and an effector of Rac, has been investigated. Arfaptin/Por (referred to as Arfaptin hereafter) was identified by the two-hybrid screen as an effector of two G proteins, Rac [12] and Arf [13]. As an effector of Rac, it was shown to interfere with the formation of actin structures at the plasma membrane that require both Rac and Arf6 [14], whereas as a partner of Arf, a G protein that regulates membrane traffic, Arfaptin, was shown to localize at the Golgi and inhibit the activation of phospholipase D by Arf in vitro [15]. Altogether, these results support the current view that Arfaptin may function in the coordination of the Rac and Arf pathways. However, whereas the specificity of Arfaptin for GTP-bound Arf has been reinforced by in vitro studies [10,16], it is yet unclear whether it acts as an actual effector of Rac [16]. In particular, Arfaptin has been reported to bind Rac with similar affinities regardless of the nature of the nucleotide, and Rac adopts the same conformation in the crystal structures of Rac–GDP–Arfaptin and Rac–GTP–Arfaptin [10].

Tiam was discovered as a metastasis-inducing GEF specific for the G protein Rac [17]. It belongs to the large family of GEFs for the Rho/Rac/cdc42 G proteins, which share a common tandem of DH and PH domains (reviewed in [18]). The crystal structure of nucleotide-free Rac bound to the DH–PH domains of Tiam [7] shows that Tiam-DH folds, as unbound

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DH domains, as a bundle of α-helices [19–22]. Its overall shape resembles a 'chaise longue' [7], with a region of conserved sequences forming the 'seat' and a more variable region forming the 'seatback'. Rac sits on both the 'seat', which interacts with the switch 1 and 2 in the vicinity of the nucleotide binding site, and the 'seatback', which interacts with the switch 2 beyond the nucleotide binding site and with the N-terminal β1 and the interswitch β2–β3 strands. This network of interactions stabilizes an open conformation of the switch 1 and a distorted conformation of the switch 2 which are incompatible with the binding of a guanine nucleotide.

The recognition of Arfaptin by both forms of Rac contradicts the prevalent view that G proteins recognize different proteins as a function of their nucleotide state. It is however reminiscent of the mechanism of GEFs, which interact with both GDP- and GTP-bound G proteins in the course of the nucleotide exchange reaction (reviewed in [23]). Their general mechanism is initiated by the formation of a low-affinity ternary complex between the GDP-bound G protein and the GEF (referred to as the ternary complex hereafter), that isomerizes to form a nucleotide-free high-affinity complex as examplified by the Rac–Tiam complex. Entry of GTP yields a low-affinity complex between the GTP-bound G protein and the GEF, and eventually dissociates the GEF from the activated G protein. The common ability of Tiam and Arfaptin to bind both nucleotide forms of Rac prompted to investigate their eventual structural similarities. Here it is reported that the GEF and the effector share a structurally superimposable domain that binds to the switch 2 of Rac with similar orientation. This domain belongs to the 'seatback' in Tiam and is contributed by both subunits of the Arfaptin dimer. The observation that it includes most of the Rac–Arfaptin interface suggests that it may constitute a docking module for the recognition of nucleotide-bound G proteins by DH domains. Multiple comparisons of unbound DH domains to Tiam using the Rac–Tiam interface as a reference suggest that the different curvatures of DH domains may reflect a functional flexibility, with the 'seatback' anchoring first before the 'seat' adjusts to promote the release of GDP. Thus, the complex of Arfaptin with Rac–GDP may be an unproductive mimic of the initial step of the exchange reaction, which has remained elusive to direct structural investigations so far.

2. Material and methods

Crystal and nuclear magnetic resonance structures were obtained from the RCSB Protein Data Bank, www.rcsb.org. Entry codes used are: Rac–Tiam: 1FOE; Arfaptin–Rac–GDP, 1I4L; Arfaptin–Rac–GDP–NH–P, 1I4T; Pix-DH, 1BY1; SOS-DH–PH, 1DBH; Vav-DH, 1F5X; Rac–GDP–GDI, 1HH4. Superpositions were done with TURBO-FRODO (Roussel A., Inisan A.-G. and Cambillau C., AFMB and BioGraphics, Marseille, France). Figures were drawn with Molscript and Raster3D.

3. Results and discussion

3.1. Arfaptin mimics the 'seatback' of DH domains

The structure of nucleotide-free Rac bound to the DH domain of the Rac–GEF–Tiam [7] was superimposed onto the Rac–GDP–Arfaptin complex [10] using, as a reference, the core domain of Rac, which has virtually the same conformation in every structure of Rac. This identified an overlapping domain in Tiam and Arfaptin that shares a common arrangement of four helices and interacts with the switch 2 of Rac (Fig. 1A,B). No such structural similarity was found with the other known complexes of Rac or those of other G protein complexes. In Tiam, it belongs to the 'seatback' and encompasses helices α1b, α2b, α4/α5 and α7, which correspond to αA and αB from monomer 2 and αB and αA from monomer 1 of Arfaptin, respectively. There is almost no sequence homology between the overlapping helices, except for residues Leu62/Gln63 in αA of Arfaptin and Ile1190/Gln1191 in α7 of Tiam which contact the interswitch and Leu67 in the switch 2 of Rac. Helix αB in monomer 1 of Arfaptin and α4/α5 in Tiam feature an equivalent kink in their middle. The domain forms extensive interactions with the switch 2 and the interswitch regions of Rac in both complexes, and contributes most of the interface of Arfaptin with Rac. In particular, the helical part of the switch 2 sits on αA and αB from the monomer 1 of Arfaptin and α4/α5 and α7 of Tiam. The similarity does not extend to the 'seat' region of Tiam, where the α1a stabilizes an open conformation of the switch 1 and α8 has the dual role of stabilizing the switch 1 and 2 and blocking the space occupied by the switch 1 in GTP-bound Rac. Arfaptin has comparatively few contacts with the switch 1 and with the nucleotide binding site. Surprisingly, the unusual open conformation of the switch 1 in the Rac–Arfaptin complexes departs from its conformations in effector-, GAP- and GDI-bound complexes [6,9,11], but is closest from its conformation in the Rac–Tiam complex.

The structural relationship of Arfaptin and Tiam is a rare example of structural mimicry in the G protein kingdom, which is reinforced by the observation that Arfaptin, as GEFs [24,25], binds with similar micromolar affinities to both GDP- and GTP-bound Rac and promotes an open conformation of the switch 1 [10]. This raises the issue of the extent to which it may behave as an actual Rac–GEF. However, unlike Tiam, Arfaptin does not interact with or distort the guanine nucleotide binding site of Rac. In addition, overexpression of Arfaptin in vivo does not induce plasma membrane ruffles that are characteristic of activated Rac [12,14], which would be expected if it functioned as a GEF for the activation of Rac. The similar binding modes of Arfaptin and Tiam to Rac are thus likely to have emerged from convergent evolution for the recognition of Rac rather than for a specialized nucleotide exchange activity, and their quaternary structures rule out that Arfaptin and DH domains are otherwise evolutionarily related.

3.2. Docking and domain closure of DH domains in the Rac–GDP-DH pre-activation complex

The structural similarity of the 'seatback' of Tiam with its counterpart in Arfaptin, where it forms most of the interface with Rac, suggests that this region may constitute a docking region for Rac per se. This was analyzed first by multiple structural comparisons of the DH domains of Tiam, Vav, Pix and SOS [7,20–22], which revealed that an overall fit of their DH domains cannot be achieved, except between Tiam and autoinhibited Vav. Instead, a significant fit can be obtained by using either their 'seats' or their 'seatbacks' behaving essentially as rigid bodies, although the detailed conformations are less well conserved in the latter case (Fig. 2A–C). Thus, the different longitudinal curvatures of the DH domains are largely due to differences in the relative orientations of their 'seat' and 'seatback' regions.

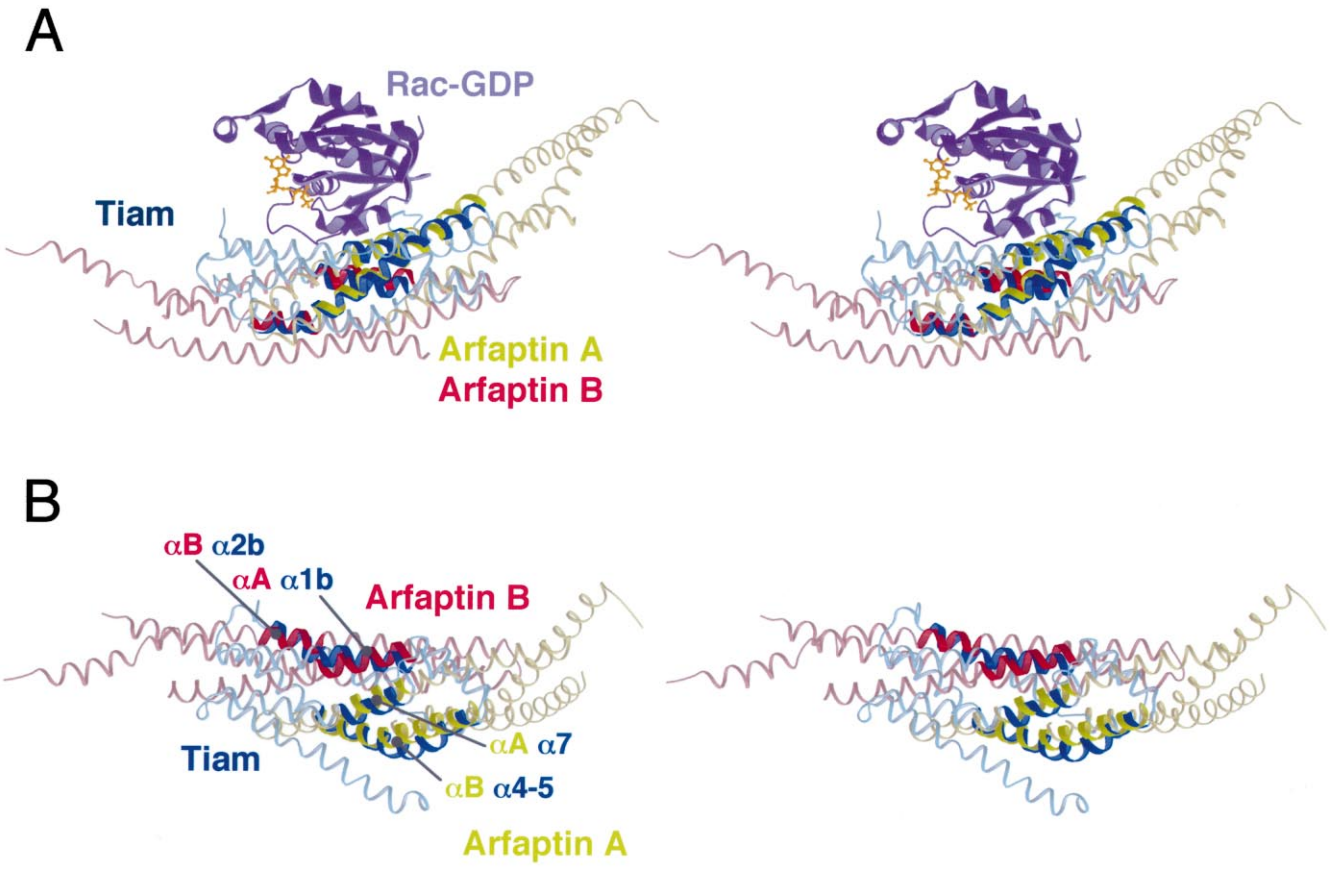

Fig. 1. Tiam and Arfaptin have a common Rac-interacting domain. A: Superposition of Rac–GTP–Arfaptin to Rac–Tiam with reference to Rac overlaps four helices (shown in brighter colors). Only Rac–GDP from the Arfaptin complex is shown for clarity. B: At 90° from view A with Rac removed. Stereo views are for direct viewing.

The closed conformation of Tiam and the open conformation of SOS and Pix may reflect a general domain closure upon binding of the DH domain to the Rac/Rho G proteins. To analyze this hypothesis, SOS-DH and Pix-DH, taken as representative of open DH conformations, were modelled in complex with nucleotide-free Rac by superposition of their 'seatback' regions onto that of Tiam in Tiam–Rac. Strikingly, superposition of the 'seatback' of unbound SOS-DH (Fig. 2D) and Pix-DH removes their 'seat' (α1a and α8) from the nucleotide binding site of Rac, in a way reminiscent of the interaction of Arfaptin with Rac. Furthermore, the orientation of the DH domains in these models is compatible with the conformation of the switch 1 region of nucleotide-bound structures of Rac (Fig. 2D), whereas Tiam in the nucleotide-free Rac–Tiam complex is not.

Altogether, these models support the hypothesis that the 'seatback' may function as a docking module for the recognition of Rac and other members of the family by DH domains, and that closure of the 'seat' domain is required to complete a productive interface. Furthermore, the observation that the open conformation of SOS and Pix can accommodate the structures of nucleotide-bound Rac suggests that the interaction of the 'seatback' of DH domains with Rac may be representative of the low-affinity pre-activation Rac–GDP–GEF complex rather than of the high-affinity nucleotide-free Rac–GEF complex. Recognition of Rac–GDP by DH domains may thus proceed by a two-step mechanism, docking first the 'seatback' onto the switch 2 and the interswitch, before the catalytic 'seat' domain reorients in order to form a large interface with Rac and dissociate GDP. Participation of the interswitch, whose sequence is more variable than that of the switch regions, may allow DH domains to discriminate between closely related G proteins before the nucleotide exchange reaction proceeds, as specific interactions are in principle optimal at this regulatory stage [26]. On the other hand, exclusion of the switch 1, whose conformation changes dramatically upon nucleotide dissociation, from the interface of the pre-activation complex may explain how DH domains solve the conflicting requirements of binding to both nucleotide-bound and nucleotide-free Rac. In agreement with a mechanism of domain closure, the hinges between the 'seat' and the 'seatback' (α1a/α1b, α2a/α2b, α4/α5 and α7/α8) align with kinks in the helices involving Pro and Gly residues that are highly conserved at +−1 positions in DH sequences (Pro1069, Gly1086, Pro1153 and Pro1189 in Tiam). A prediction of the docking/domain closure mechanism is that mutation of residues in the hinges should alter the GEF activities of DH domains, and that the 'seatback' and the 'seat' should be responsible, to some extent, of binding to Rho/Rac G proteins and catalysis of nucleotide exchange, respectively.

4. Conclusion

Multiple comparisons of the Arfaptin–Rac-nucleotide complexes to structures of bound and unbound DH domains identified a minimal domain found in Arfaptin and DH domains

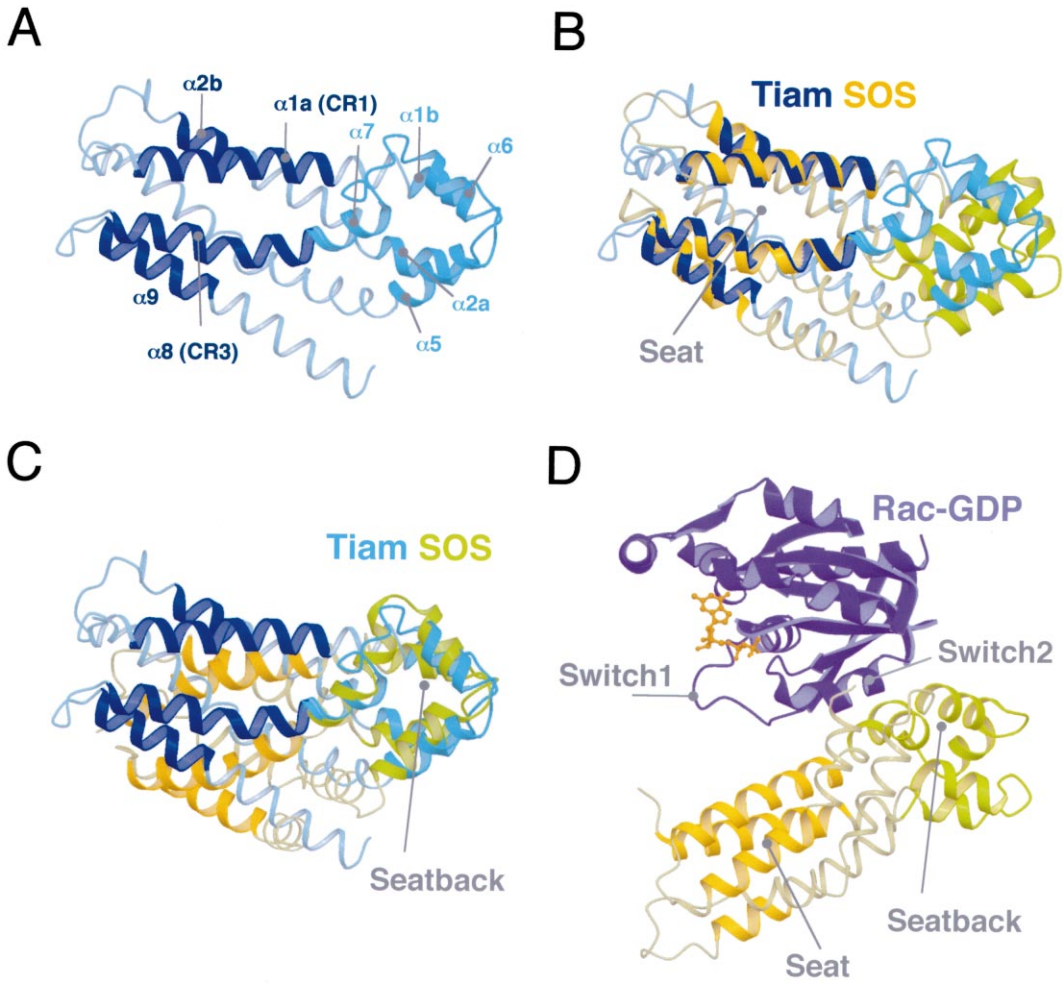

Fig. 2. Modelling the docking and domain closure of DH domains. A: Structural definition of the 'seat' (blue) and 'seatback' (cyan) regions of Tiam-DH as derived from the superpositions of the DH domains of Tiam, SOS, Pix and Vav. B: Superposition of the 'seats' of Tiam-DH (blue and cyan) and SOS-DH (yellow and green). C: Superposition of the 'seatbacks' of Tiam-DH and SOS-DH. D: Model of the ternary Rac–GDP-DH complex. Docking of the 'seatback' of SOS-DH onto Rac–GDP was obtained by superpositions of SOS-DH on the 'seatback' of Tiam-DH and of Rac–GDP from the Arfaptin complex on nucleotide-free Rac, both from the Tiam–Rac complex. The PH domain of SOS has been removed for the superposition, but would conflict with Rac otherwise. Orientations in A–C are as in Fig. 1B; orientation in D is as in Fig. 1A.

that may function as a docking module for the recognition of Rac. This resemblance allowed to model the pre-activation Rac–GDP-DH domain complex, which suggests that DH domains may function by a two-step anchoring/domain closure mechanism. Thus, DH domains would take on part of the large conformational changes that are required to yield a productive nucleotide-free intermediate. Interestingly, domain closure of Sec7 Arf–GEFs [27] and an anchoring mechanism for cdc25 Ras–GEFs [28] have been suggested recently, which, as for DH domain, now await structural characterization.

The structural resemblance of Arfaptin to a Rac–GEF suggests that Arfaptin–Rac–GDP mimics a unproductive pre-activation GEF–Rac–GDP complex, but it does not clarify what could be its role as a partner that binds to both nucleotide states of Rac. The possibility that Rac is not a classical GDP/GTP molecular switch is ruled out by its cellular functions [3]. In addition, Rac does not depart from other G proteins in that it adopts many different conformations by interacting with its various partners [6–11]. The open question is whether Arfaptin defines a novel class of G protein partners that scratches the molecular switch concept, or if a more conventional function is still to be discovered, possibly through the mediation of an unknown protein.

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