Dissociation of GDP Dissociation Inhibitor and Membrane Translocation Are Required for Efficient Activation of Rac by the Dbl Homology-Pleckstrin Homology Region of Tiam*

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Small G proteins of the Rho/Rac/Cdc42 family are associated with lipid membranes through their prenylated C termini. Alternatively, these proteins form soluble complexes with GDI proteins. To assess how this membrane partitioning influences the activation of Rac by guanine nucleotide exchange factors, GDP-to-GTP exchange reactions were performed in the presence of liposomes using different forms of Rac-GDP. We show that both non-prenylated Rac-GDP and the soluble complex between prenylated Rac-GDP and GDI are poorly activated by the Dbl homology-pleckstrin homology (DH-PH) domain of the exchange factor Tiam1, whereas prenylated Rac-GDP bound to liposomes is activated about 10 times more rapidly. Sedimentation experiments with liposomes reveal that the DH-PH region of Tiam1 forms, with nucleotide-free prenylated Rac, a membrane-bound complex from which GDI is excluded. Taken together, these experiments demonstrate that the dissociation of Rac-GDP from GDI and its translocation to membrane lipids favors DH-PH-catalyzed nucleotide exchange because the steric hindrance caused by GDI is relieved and because the membrane environment favors functional interaction between the DH-PH domain and the small G protein.

Small G proteins of the Rho family, including Rho, Rac, and Cdc42p, undergo two interdependent cycles. First, they cycle between inactive (GDP) and active (GTP) conformations through the catalytic action of guanine exchange factors (GEF) and GTPase-activating proteins. Second, they cycle between cytosolic and membrane-associated forms through the action of GDI proteins (1–3). How these two cycles are coupled is critical for the proper interaction of Rho proteins with their targets. It is generally assumed that Rho proteins must be in the GTP conformation and associated to membranes to trigger a cellular response.

Dbl homology (DH) domains catalyze the exchange of guanine nucleotides on small G proteins of the Rho family (4, 5). The DH domain is an all-α-helix fold that is systematically followed by a pleckstrin homology (PH) domain. The DH-PH tandem is thus the hallmark of Rho GEFs, which otherwise display variable domain composition and organization. Recent structural and mutagenesis studies on DH-PH domains, either isolated or in complex with Rho proteins, have given insights into the mechanism by which these domains promote the release of the bound nucleotide (6–10). One of the best-studied examples is Tiam, a GEF for Rac. The DH domain of Tiam makes extensive contacts with the switch I and II regions of Rac and modifies the magnesium, sugar, and guanine base-binding regions to destabilize the bound nucleotide (9). In addition, structure-based mutagenesis studies show that the specificity between functional GEF/Rho pairs relies on a few residues at the DH/switch II interface (11–13). Binding to switch I and II regions is a general property of GEFs for small G proteins (14).

Despite these spectacular progresses, one aspect of the activation of Rho proteins by DH domains remains obscure: how does the nucleotide exchange reaction accommodate with the interaction of Rho proteins with membrane lipids or with GDI proteins? Rho proteins contain a geranyl-geranyl group at their C termini. This C20 branched isoprene has a high lipid/water partition coefficient and anchors Rho proteins on lipid membranes (15). On the other hand, GDI proteins solubilize Rho proteins in the cytosol by shielding the geranyl-geranyl group from the solvent (2, 16–18). The GDI/Rho interaction is based on two contacts: the isoprene group inserts into a deep pocket made by the immunoglobulin-like β sandwich of GDI and the switch I and II regions of Rho interact with the regulatory arm of GDI, a small helix-loop-helix motif (18). The latter interaction makes the Rho/GDI interaction partially dependent on the conformation of Rho. Thus GDI interacts preferentially with the GDP-bound form of Rho proteins (19), although equal interactions with the GDP- and GTP-bound forms have been reported in some cases (20, 21). Consequently, Rac-GDP is generally found in complex with GDI in the cytosol, whereas Rac-GTP is preferentially associated with membranes (2, 3, 22).

Overall, the structure of nucleotide-free Rac in complex with the DH-PH region of Tiam1 and that of prenylated Cdc42-GDP in complex with GDI show a strong overlap of the interacting regions, particularly at the level of the switch I and II regions (9, 18). This strongly suggests that the two interactions are mutually exclusive and consequently that Rho-GDP must be released from GDI before being activated by the DH-PH module. However, given the bipartite nature of the Rho/GDI interface, the formation at early stages of the exchange reaction of a ternary complex, where GDI interacts only with the prenyl group of Rho, leaving the switch regions accessible for the exchange factor, cannot be excluded. Schematically, two opposite models have been proposed for GEF-catalyzed nucleotide exchange on Rho proteins coupled to membrane translocation (1, 2, 3). In the first model, the GEF acts on the cytosolic...
complex between Rho-GDP and GDI. After nucleotide exchange, Rac-GTP translocates to membranes. In the second model, membrane translocation precedes GEF action: Rac-GDP dissociates from GDI and translocates to membranes; then GEF promotes GDP-to-GTP exchange on membrane-bound Rac. As a first step toward understanding the sequence of events that, from soluble GDI-Rac-GDP complex, lead to membrane-bound Rac-GTP, we have studied the catalysis of nucleotide exchange on geranyl-geranylated Rac by the DH-PH region of Tiam1 in the presence of GDI and liposomes. Our results demonstrate that dissociation of GDI and membrane translocation is a prerequisite for the efficient activation of Rac by a DH-PH domain.

EXPERIMENTAL PROCEDURES

Purification of the Rac-RhoGDI Complex (Saccharomyces cerevisiae Expression)—Constructions of Human Rac1 with an N-terminal His6 tag and RhoGDI-1 with an N-terminal FLAG cloned in the yeast 2µ shuttle vector YepPgal, were a gift from R. Nakamoto (23, 24). These constructs were transformed in S. cerevisiae Y1 strain (MATa, ura3-52, leu2-3,112,his4-619,sec6-4,GAL) and selected for leucine/uracil independence. Transformed yeast cells were grown in a 5-liter fermenter. After cell wall lysis and fractionation, the Rac1-RhoGDI complex was purified from the cytosol as described for the RhoA-GDI complex using two sequential affinity chromatography assays on a TALON metal affinity resin column (Clontech) and an M2-anti-FLAG antibody column (Sigma Chemical) (23, 24). About 100 µg of Rac1-RhoGDI complex (95% purity) was obtained from one fermenter. The Rac1-RhoGDI GDP complex was stored at −80°C in 25 mM Tris, pH 8, 100 mM NaCl, 2 mM MgCl2, 10% glycerol, and 2 µM GDP.

Purification of C-terminally Truncated Rac1 and Tiam1 DH-PH—Full-length human Rac1 was cloned in a PGEX-T vector. The pProEX-Tag and RhoGDI-1 with an N-terminal FLAG cloned in the yeast 2µ expression construct were a gift from J. Sondek (9). GST-Rac1 and His6-Tiam1 fragment were purified by affinity chromatography on glutathione-agarose (Amersham Biosciences) or Ni2+-agarose beads (QIAGEN) respectively according to the manufacturer procedures. GST from the rac chimera was removed by cleavage with thrombin. All constructs were sequence-proofed. The purity of proteins was assessed by Coomassie Blue-stained SDS-PAGE, and the protein concentration was estimated by a protein assay (Bio-Rad).

Azoletin Liposomes—Large unilamellar liposomes made of unpuriﬁed soybean phospholipids (azolectin type IIS; Sigma Chemical) were prepared by the reversed-phase method (25). Azoletin (20 mg) was dissolved in 6 ml diethylther in a 100-ml round-bottomed flask. One milliliter of buffer A (20 mM HEPES, pH 7.5, and 180 mM sucrose (isomotic with 1 M NaCl)) was added and the two-phase system was set back at 1°C for 1–2 min in a water bath sonicator to make an emulsion. The solvent was slowly evaporated in a rotary evaporator (100 rpm) under moderate vacuum at 20–25°C. After 45 min, the liposome suspension was collected in an Eppendorf tube and was further incubated in a vacuum chamber for 15 min to eliminate traces of ether and completed up to 1 ml with buffer A. The liposome suspension was frozen in liquid nitrogen and stored at −80°C. Liposomes were extruded before use through 0.4-µm pore-size polycarbonate filters (Millipore). The suspension was diluted ﬁve times in buffer B (20 mM HEPES, pH 7.5, and 150 mM NaCl) to dilute the excess sucrose solution. The liposomes were collected by centrifugation in a TL100 Beckman centrifuge at 400,000 × g, 18°C, for 25 min and resuspended in buffer B.

Sedimentation Assay—Preynlated Rac-GDP in complex with GDI was incubated for 20 min at 30°C with sucrose-loaded azoletin liposomes (20 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM MgCl2, 1 mM dithiothreitol (buffer C) in a final volume of 80 µl. Nucleotides (50 µM ﬁnal concentration of GDP-Mg or GTP-S-Mg) were added as indicated. When indicated, magnesium was chelated by the addition of 2 mM EDTA (1 mM free Mg2+) and alkaline phosphatase (Sigma; ﬁnal concentration, 180 units/ml) was added to hydrolyze guanine nucleotides. After incubation, vesicles were recovered by centrifugation at 400,000 × g, 24°C, for 25 min. The pellet was resuspended in the same volume of buffer, and the amount of preynlated Rac and Tiam1 in the pellet and in the supernatant was determined by densitometry after protein separation by SDS-PAGE and Coomassie Blue staining.

GDI Removal Protocol—The Rac-GDP-GDI complex (1.0 µM) was incubated at 30°C for 30 min with azoletin liposomes (5 mg/ml) in buffer C supplemented with 2 mM EDTA and 50 µM GTP. Liposomes and bound proteins were collected by centrifugation (400,000 × g, 25 min, 18°C) and washed one time by resuspension/centrifugation in buffer C. The final liposome suspension containing liposome-translocated RacGTP was incubated for 30 min at room temperature to promote GTP hydrolysis.

Nucleotide Binding Assay—The binding of [35S]GTP–S to Rac was measured as described by Franco et al. (26) for Arf1. Briefly, the Rac1-GDI-GDI complex was incubated at 30°C with [35S]GTP–S (20 µM, 2000 cpm/pmol) in the presence of azoletin liposomes (0 to 3 mg/ml) in buffer C. At the indicated times, 20-µl aliquots were removed, diluted into 2 ml of ice-cold buffer (buffer D; 20 mM HEPES, pH 7.5, 100 mM NaCl, and 10 mM MgCl2), and filtered on nitrocellulose filter discs (Schleicher & Schuell). Filters discs were washed twice with 2 ml of buffer D, dried, and counted. The same protocol was used to measure the binding of [35S]GTP–S to Rac-GDP bound to liposomes and on unprenylated Rac in solution or in the presence of liposomes.

GTase Assay—Unprenylated Rac-GDP (0.8 µM) or preynlated Rac-GTP bound to liposomes (see the GDI removal protocol) was incubated at room temperature for 15 min in buffer C with 2 mM EDTA (1 µM free MgCl2) and with [γ–32P]GTP (10 µM). The GTase reaction was initiated by the addition of 2 mM MgCl2 (1 mM free MgCl2). At the indicated times, 20-µl aliquots were removed, diluted into 2 ml of ice-cold buffer D, and counted on nitrocellulose filter as described for nucleotide binding.

RESULTS

Reconstitution of the GTP-dependent Translocation of Rac to Liposomes—We purified recombinant geranylgeranylated Rac-GDP from yeast cells as a stoichiometric complex with RhoGDI-1 (GDI) according to the protocol developed by Read et al. (23, 24) for the complex between RhoA-GDI and GDP (Fig. 1). We ﬁrst assessed the ability of Rac-GDP, initially associated with GDI, to interact with membrane lipids and undergo GDP to GTP exchange. To this end, we incubated the complex between Rac-GDP and GDI with radiolabeled GTP–S and with liposomes made from soybean lipids (azolectin). Because GDI strongly inhibits the spontaneous release of GDP from Rho proteins, incubations were performed at low Mg2+ concentration (1 µM) to accelerate nucleotide exchange. At the end of the incubation, the concentration of free Mg2+ was set back at 1 mM. The binding of GTP–S to Rac was determined by a ﬁltration assay, and the membrane partitioning of Rac and GDI was determined by separating the soluble proteins and the liposome-bound proteins by centrifugation. Fig. 2B shows that the extent of GTP–S binding on Rac strongly increased with the liposome concentration. Notably, no detectable binding of GTP–S was observed in the absence of membrane lipids (Fig. 2B, open circles). Fig. 2A shows that in all incubations with GTP–S GDI remained essentially soluble, whereas the amount of membrane-bound Rac increased with liposome concentration. In contrast, in the presence of GDP, Rac remained essentially soluble as a complex with GDI. However, a fraction of Rac-GDP bound to liposomes could be observed at high liposome concentration (> 1 mg/ml) (Fig. 2A). Note a slight contamination of the pellet by soluble GDI at increasing liposome concentration caused by the increase in the volume of the pellet. A slight contamination of the pellet with soluble Rac could also have occurred but this could not account for the full Rac signal in the pellet. These experiments show that one can reconstitute on liposomes the GTP-dependent translocation of Rac to membrane lipids starting from a soluble Rac-GDP-GDI complex.

Tiam-catalyzed GDP/GTP–S Exchange on Rac-GDP in Complex with GDI and in the Presence of Liposomes—Next, we studied the inﬂuence of liposomes on the kinetics of Tiam1-catalyzed nucleotide exchange on Rac-GDP, initially in complex with GDI, at physiological (1 mM) Mg2+ concentration. We did not observe any signiﬁcant GDP-to-GTP–S exchange when the complex was incubated in the absence of liposomes with or without Tiam (Fig. 3A). In contrast, in the presence of azoletin
liposomes, both spontaneous and Tiam-catalyzed GDP-to-GTP exchange on Rac could be detected (Fig. 3, B and C). Interestingly, increasing liposome concentration had two effects: it accelerated the initial rate of the reaction and increased the amount of Rac-GTP that formed at equilibrium. The second effect resembles that observed at low magnesium concentrations in the absence of Tiam (see Fig. 2B) and may reflect the opposite mass action effects of liposomes and GDI on the balance between the GTP- and the GDP-bound forms of Rac. More surprising is the effect of liposome concentration on the initial rate of GTP\(\gamma\)S binding. Insofar as only Rac-GDP was present initially, an increase in the initial rate of GTP\(\gamma\)S binding must reflect a positive effect of liposomes on the ability of Rac-GDP to undergo spontaneous or Tiam-catalyzed nucleotide exchange. At first glance, this does not seem compatible with the fact that Rac-GDP is essentially soluble and in complex with GDI. However, because sedimentation experiments revealed that a small amount of Rac, bound to liposomes and devoid of GDI, could be detected in incubations conducted in the presence of GDP (Fig. 2A), we suspected that this fraction of Rac could be a better substrate for Tiam-catalyzed nucleotide exchange than Rac-GDP in complex with GDI.

A GDI-release Protocol That Produces Liposome-bound Rac-GDP and Facilitates Rac Activation by Tiam—We reasoned that if Rac-GDP bound to liposome was more readily activated by Tiam than soluble Rac-GDP in complex with GDI, one should change dramatically the kinetics of GDP-to-GTP exchange by modifying the partitioning of Rac-GDP. The second effect resembles that observed at low magnesium concentrations. The balance between the GTP- and the GDP-bound forms of Rac was sensitive to the presence of liposome concentrations in the absence of Tiam (see Fig. 2A). The second effect resembles that observed at low magnesium concentrations, and may reflect the opposite mass action effects of liposomes and GDI on the balance between the GTP- and the GDP-bound forms of Rac.

Membrane Localization Potentiates the Exchange Activity of Tiam1 DH-Ph on Rac—Next, we wished to compare liposome-bound prenylated Rac-GDP with soluble non-prenylated Rac-GDP for their responsiveness to the exchange activity of Tiam DH-Ph. This should allow us to assess the effect of the membrane colocalization of Rac-GDP and Tiam1 DH-Ph on the
nucleotide exchange reaction per se (i.e., independently of the effect of GDI on Rac). Indeed, besides being required for the dissociation of prenylated Rac from GDI, liposomes may also positively or negatively influence the interaction between Rac-GDP and Tiam DH-PH by effects such as reduction of dimensionality (liquid volume versus membrane surface) or conformational change.

GDP-to-GTP exchange reactions on non-prenylated Rac-GDP were conducted in solution at various Tiam1 DH-PH concentration (Fig. 5C). Importantly, azolectine liposomes had no effect on the reaction (Fig. 5D). The apparent rate constant was plotted as a function of Tiam DH-PH concentration and was compared with that observed on liposomes-bound Rac-GDP (Fig. 5E). Remarkably, the DH-PH region of Tiam1 was 10 to 20 times more active on prenylated Rac-GDP bound to membrane lipids than on soluble non-prenylated Rac-GDP. The comparison between these two forms was made easy by the fact that both underwent similar spontaneous exchange kinetics in the absence of exchange factor. The maximal exchange activity \( k_{\text{cat}} \) of Tiam DH-PH on Rac-GDP bound to liposomes could not be determined because we could not resolve the kinetics of GDP/GTP exchange at saturating DH-PH concentration. However, from a rough extrapolation of the data, one can estimate that there is a difference of 1 to 2 orders of magnitude in the maximal exchange activity (Fig. 5E). We conclude from these experiments that colocalization at a lipid membrane surface strongly favors the nucleotide exchange activity of the DH-PH domain of Tiam toward Rac.

**Formation of a Liposome-bound and Nucleotide-free Complex between Rac and Tiam DH-PH**—The above experiments suggest that the DH-PH domain of Tiam acts preferentially on prenylated Rac-GDP associated with membrane lipids. If this assessment is correct, catalytic intermediates of the nucleotide exchange reaction should be found also associated with membrane lipids. The essential intermediate of the catalysis of nucleotide exchange on G protein by GEFs is a nucleotide-free G protein-GEF complex. We assessed the membrane partitioning of such a complex in liposome sedimentation experiments. Prenylated Rac-GDP in complex with GDI (0.8 M) and Tiam DH-PH (0.3 M) were incubated with liposomes. To favor the stabilization of the nucleotide-free complex, incubations were performed at low Mg\(^2+\) (2 mM EDTA) in the presence of alkaline phosphatase, which hydrolyzes GDP as it dissociates from Rac. As shown in Fig. 6, the DH-PH domain alone of Tiam was more than 50% soluble (lane 3) although a significant amount (30%) was associated with liposomes. However, after incubation with prenylated Rac, initially in the GDP-bound form and complexed to GDI but under conditions that favor the formation of the nucleotide-free form of Rac, the amount of membrane-bound Tiam DH-PH increased 2.5-fold (lane 2). Rac was also found to be almost completely associated with liposomes, whereas GDI remained in the supernatant (lanes 2 and 3).
Importantly, no cotranslocation of Rac and Tiam DH-PH to liposomes was observed in a control incubation conducted in the presence of GDP and in the absence of EDTA and alkaline phosphatase (lane 1). We conclude from these experiments that the nucleotide-free complex between prenylated Rac and Tiam DH-PH is a membrane-bound complex from which GDI is excluded.

**DISCUSSION**

With the notable exception of Ran, most small G proteins interact with lipid membranes at some steps of the GDP/GTP cycle. In many cases, the interconversion between GDP- and GTP-bound forms is directly or indirectly coupled to the translocation of the protein between the cytosol and membranes, making the catalysis of GDP to GTP exchange by GEFs a complex issue. In Arf1, the coupling is direct: a conformational change in the myristoylated N-terminal helix of Arf1 strengthens its interaction with membrane lipids and is required for GEF-catalyzed GDP-to-GTP exchange (27, 28). In Rho proteins, the coupling is indirect. The GDP- and GTP-bound forms display the same avidity for membrane lipids, but Rho-GDP is more cytosolic because of its preferential interaction with GDI (19; see, however, Ref. 20). The sequence of events that leads, starting from Rho-GDP in a cytosolic complex with GDI, to membrane anchored Rho-GTP is not well understood (1). It has been suggested that exchange factors act on the soluble GDI-Rho-GDP complex and that after nucleotide exchange, Rho-GTP dissociates from the tripartite complex and translocates to membranes (24, 29). Alternatively, the dissociation of GDI and the membrane translocation of the GDP-bound form may precede or accompany the functional interaction of Rho with GEF (22). Here, we have taken advantage of a recently described overexpression system for prenylated Rho proteins in complex with GDI (24) to explore the nucleotide exchange reaction on the small G protein Rac in a minimal system with liposomes.

To reconstitute the GDP/GTP switch of Rac on liposomes, one faces a dilemma. To handle prenylated Rac easily, the protein must be purified in complex with GDI. In that case, however, Rac-GDP remains essentially associated with GDI in solution even after incubation with a large amount of liposomes (Fig. 2). Alternatively, detergents may be used during the purification procedure to dissociate Rac-GDP from GDI. However, this will make the reconstitution with liposomes difficult. The group of
Fig. 6. A liposome-bound and nucleotide-free complex between prenylated Rac and Tiam1 DH-PH domain. Prenylated Rac-GDP in complex with GDI (0.8 μM) and Tiam DH-PH (0.3 μM) were incubated for 30 min at 30 °C with 1.5 mg/ml azolectin liposomes. When indicated, EDTA (to obtain 1 μM free Mg²⁺), alkaline phosphatase (AP; to hydrolyze free nucleotides) or GDP (20 μM) were added. After incubation, the samples were centrifuged. The supernatant (S) and the liposome pellet (P) were analyzed by SDS-PAGE, Coomassie Blue staining and densitometry. The percentage of liposome-bound proteins is indicated in the bottom graph.

Takai (1, 30) reported interesting observations on the effects of both GDI and prenylation of Rho/Rac proteins on the catalysis of guanine nucleotide exchange, but the influence of detergent and lipids in the reaction was not appreciated and thus prenylation was considered to participate directly in the interaction of Rho/Rac proteins with exchange factors. To overcome these difficulties, we devised a protocol whereby, starting from Rac-GDP in complex with GDI, we recapitulated a complete cycle of nucleotide exchange and GTP hydrolysis to get prenylated Rac-GDP bound to liposomes and devoid of GDI (Fig. 4). This allows us to establish an elementary scheme for the GDI and DH-PH-dependent activation of Rac at the lipid surface.

In solution, the DH-PH domain of Tiam was unable to promote GDP-to-GTP exchange on prenylated Rac-GDP in complex with GDI (Fig. 3A). In sharp contrast, robust exchange activity was observed on prenylated Rac-GDP that was bound to azolectin liposomes (Fig. 5B). Starting from the Rac-GDP-GDI complex, the exchange rate increases with lipid concentration, and this correlates with the appearance of a small fraction of Rac-GDP associated with liposomes and dissociated from GDI (Figs. 2 and 3). Last, sedimentation experiments revealed the formation of a liposome-bound complex between nucleotide-free Rac and the DH-PH domain of Tiam1, from which GDI was excluded (Fig. 6). These four observations exclude the formation of a functional tripartite complex between Rac, GDI, and DH-PH in solution and favor a two-step mechanism. First, Rac-GDP dissociates from GDI and translocates to membrane lipids. Second, DH-PH catalyzes the replacement of GDP by GTP on Rac anchored at the membrane surface. Although these two steps may be functionally coupled, they are mechanistically different. In the cell, membrane translocation of Rac can occur even with a catalytically inactive form of the exchange factor Vav, suggesting that there is a mechanism that controls the membrane recruitment of Rac before the nucleotide exchange reaction (31).

One important finding is that the DH-PH module of Tiam1 displays a much better activity on prenylated Rac-GDP bound to liposomes (Fig. 5B) than on soluble unprenylated Rac-GDP (Fig. 5C). Because the two forms display the same rate of spontaneous GDP release (compare Fig. 5, B and C), it is unlikely that the difference in DH-PH-catalyzed nucleotide exchange arises from differences in the conformation of Rac. One explanation for the catalytic advantage provided by membrane localization is reduction of dimensionality: the lipid membrane acts as a template that concentrates and better orients the small G protein and the DH-PH module, hence favoring their mutual interaction. Because both Rac and the DH-PH module have elements permitting membrane attachment (prenylation and PH domain), this mechanism is likely to contribute to the effects observed here. However additional mechanisms may play a role. If membranes solely concentrated the proteins, the maximal activities at saturating concentration (k_cat) should be similar in solution and in the presence of liposomes. However, extrapolation of the dose response curves shown in Fig. 5E suggests different k_cat, implying that membrane colocalization favors the catalytic mechanism per se. One recurrent observation on DH-PH domains is that their activity on non-prenylated Rho proteins in solution is very weak compared with other catalytic domains that promote nucleotide exchange on small G proteins (discussed in Ref. 32). This raised the possibility that the DH-PH module in solution may be somehow autoinhibited. Because the structures of DH-PH modules show great variations in the relative orientation of the DH and PH domains, it is tempting to assume that membrane binding of the PH domain may favor structural rearrangements at the interface with the DH domain that impact on the nucleotide exchange activity (4, 10). If so, membrane-bound prenylated Rac-GDP should be in a better position to detect membrane-induced changes in the DH-PH module than soluble unprenylated Rac-GDP. Notably, we did not observe any effect of liposomes on the nucleotide exchange activity of Tiam1 DH-PH on soluble unprenylated Rac (Fig. 5D).
It has been shown recently that the PH domain of Tiam1 interacts preferentially with phosphatidylinositol 3-phosphate but that this interaction had no effect on the activity of Tiam1 on unprenylated Rac in solution (33). It will be interesting to assess the effect of phosphoinositides on nucleotide exchange reactions performed in the presence of liposomes of defined lipid composition and with prenylated Rac. In addition, it should be noted that the membrane localization of these domains may thus control the proper interaction of the GTPase cycle of Rac on liposomes should help to define the molecular mechanisms by which the activation of this small G protein is regulated.

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