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Inhibition of the Action of a Stimulatory GDP/GTP Exchange Protein for *smg* p21 by Acidic Membrane Phospholipids

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A stimulatory GDP/GTP exchange protein for *smg* p21 (*smg* p21 GDS) stimulated the dissociation of GDP from *smg* p21B. This reaction was inhibited by acidic membrane phospholipids such as phosphatidylinositol, phosphatidylinositol-4-monophosphate, phosphatidylinositol-4,5-bisphosphate, phosphatidic acid, and phosphatidylserine but not by phosphatidylcholine or phosphatidylethanolamine. These acidic phospholipids inhibited the *smg* p21 GDS action in a manner competitive with both *smg* p21 GDS and *smg* p21B. *smg* p21 GDS has other actions to inhibit the binding of *smg* p21B to membranes and to induce the dissociation of prebound *smg* p21B from the membranes. The acidic phospholipids also inhibited these two actions of *smg* p21 GDS. *smg* p21B has a polybasic region and an isoprenoid moiety in its C-terminal region which are necessary for its membrane-binding activity and its sensitivity to the *smg* p21 GDS actions. Therefore, it is possible that acidic membrane phospholipids interact with this polybasic region and thereby inhibit the *smg* p21 GDS actions.

Key words: Small GTP-binding protein — *smg* p21 — GDP/GTP exchange protein — Acidic phospholipids

The *smg* p21 family, composed of A and B members, belongs to a *ras* p21/*ras* p21-like small G protein[2] superfamily.[1-9] *smg* p21s have the same putative effector domain as *ras* p21s and could exert actions similar or antagonistic to those of *ras* p21s.[1-5] In fact, the K*rev*-1 gene has been shown to suppress the transforming activity of the activated Ki-*ras* p21 in NIH/3T3 cells.[4] *smg* p21s have two interconvertible forms, GDP-bound inactive and GTP-bound active forms; the conversion from the GDP-bound to the GTP-bound form is regulated by a stimulatory GDP/GTP exchange protein, named *smg* p21 GDS, and the reverse conversion is regulated by GTPase activating protein, named *smg* p21 GAP.[8-12] The rate-limiting step of the GDP/GTP exchange reaction is the dissociation of GDP from *smg* p21s, and *smg* p21 GDS stimulates this reaction and enhances the subsequent binding of GTP to *smg* p21s.[12] *smg* p21B has a polybasic region and a geranylgeranyl moiety in its C-terminal region through which *smg* p21B interacts with membranes and *smg* p21 GDS.[13,14] The binding of *smg* p21B to membranes is reversible and this binding is regulated by *smg* p21 GDS: *smg* p21 GDS inhibits the binding of *smg* p21B to membranes and induces the dissociation of prebound *smg* p21B from the membranes.[15] Moreover, *smg* p21B is phosphorylated by protein kinase A at the serine residue just downstream of the polybasic region in the C-terminal region.[16-18] This phosphorylation renders *smg* p21B sensitive to the *smg* p21 GDS actions to stimulate the GDP/GTP exchange reaction, to inhibit the binding of *smg* p21B to membranes, and to induce the dissociation of prebound *smg* p21B from the membranes.[18] It is likely from these observations that the polybasic region of *smg* p21B may interact with the acidic polar head groups of the membrane phospholipids and thereby facilitate the binding of *smg* p21B to membranes. In this study, therefore, we have examined the effect of various membrane phospholipids on *smg* p21B and *smg* p21 GDS.

Total lipids extracted from bovine brain by chloroform/methanol[19] affected neither the GTPγS-binding activity of *smg* p21B nor its GTPase activity but inhibited the *smg* p21 GDS action to stimulate the dissociation of [^{3}H]GDP from *smg* p21B (Table I). In these experiments, *smg* p21B purified to near homogeneity from human platelet membranes[20] and *smg* p21 GDS purified to near homogeneity from an overexpressing *E. coli* strain[21] were used. Total lipids were next separated

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[2] Abbreviations used are: G proteins, GTP-binding proteins; GDS, GDP dissociation stimulator; GAP, GTPase activating protein; protein kinase A, cyclic AMP-dependent protein kinase; GTPγS, guanosine 5′-(3-*O*-thio)triphosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol-4-monophosphate; PIP_2, phosphatidylinositol-4,5-bisphosphate; PA, phosphatidic acid; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

into phospholipids, glycolipids, and neutral lipids.[22] When the effect of each lipid on the *smg* p21 GDS action was examined, only phospholipids inhibited the *smg* p21 GDS action (Table I). Among various phospholipids, acidic phospholipids including PI, PIP, PIP_2, PA, and PS inhibited the *smg* p21 GDS action in a dose-dependent manner, whereas PC and PE were ineffective (Fig. 1). The efficiencies of PI, PIP, PIP_2, and PA were similar and higher than that of PS. PI and PA inhibited the *smg* p21 GDS action in a manner competitive with both *smg* p21 GDS and *smg* p21B (Fig. 2). Moreover, PI and PA

Table I. Effect of Various Lipids on the [^{35}S]GTPγS-Binding and GTPase Activities of *smg* p21B and the *smg* P21 GDS Action

	[^{35}S]GTPγS-binding activity[a] (%)	GTPase activity[b] (%)	*smg* p21 GDS action[c] (%)
Control	100[d]	100	100
Total lipids[e]	100	100	87
Phospholipids	100	100	68
Glycolipids	100	100	100
Neutral lipids	100	100	100

a) The [^{35}S]GTPγS-binding activity of *smg* p21B (2 pmol) was assayed as described.[1]
b) The GTPase activity of *smg* p21B (2 pmol) was assayed as described.[1]
c) The *smg* p21 GDS action to stimulate the dissociation of [^{3}H]GDP from *smg* p21B (2 pmol) was assayed in the presence of *smg* p21 GDS (6 pmol) as described.[12]
d) The [^{35}S]GTPγS-binding and GTPase activities of *smg* p21B and the *smg* p21 GDS action are expressed as percent relative to the activities in the absence of lipids.
e) Each lipid was used for the assays in an amount of 50 μg/ml.

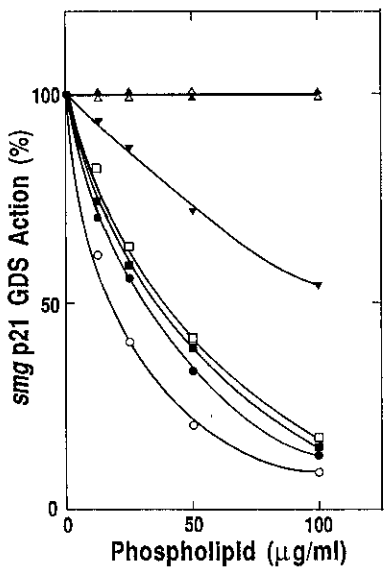

Fig. 1. Effect of various phospholipids on the *smg* p21 GDS action. The *smg* p21 GDS action to stimulate the dissociation of [^{3}H]GDP from *smg* p21B was assayed in the presence of the indicated amounts of phospholipids and fixed amounts of *smg* p21B (2 pmol) and *smg* p21 GDS (6 pmol) as described.[12] (●), PI; (□), PIP; (■), PIP_2; (○), PA; (▼), PS; (▲), PC; (△), PE. The *smg* p21 GDS action was expressed as percent relative to the activity in the absence of phospholipid. The results are representative of three independent experiments.

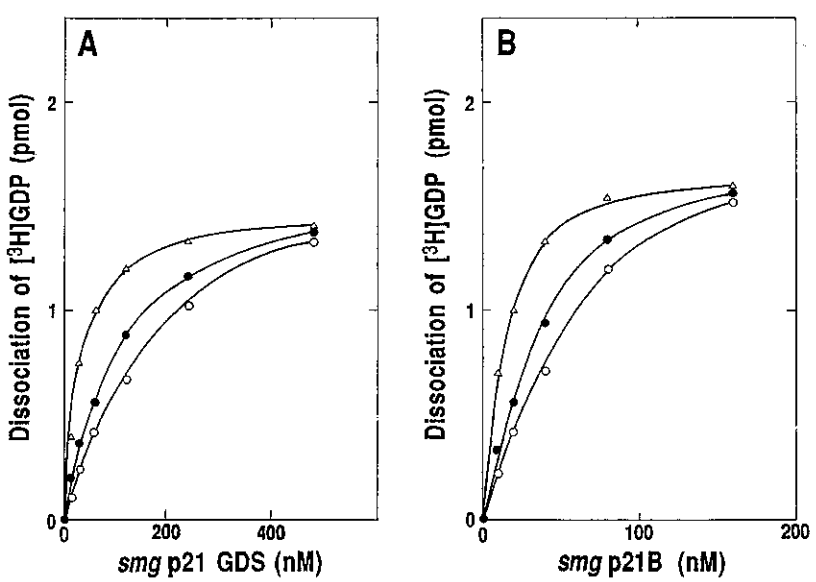

Fig. 2. Inhibition by PI and PA of the *smg* p21 GDS action. The *smg* p21 GDS action to stimulate the dissociation of [^{3}H]GDP from *smg* p21B was assayed in the presence of 25 μg/ml of PI or PA as described.[12] A, with the indicated amounts of *smg* p21 GDS in the presence of the fixed amount of *smg* p21B (2 pmol); B, with the indicated amounts of *smg* p21B in the presence of the fixed amount of *smg* p21 GDS (6 pmol). (●), in the presence of PI; (○), in the presence of PA; (△), in the absence of phospholipid. The results are representative of three independent experiments.

Table II. Effect of PI and PA on the *smg* p21 GDS Actions to Inhibit the Binding of *smg* p21B to Erythrocyte Ghosts and to Induce the Dissociation of Prebound *smg* p21B from the Erythrocyte Ghosts

	−GDS		+GDS	
	Membrane fraction (%)	Soluble fraction	Membrane fraction (%)	Soluble fraction
Binding of *smg* p21B[a]				
Control	99[b]	1	27	73
PI[c]	99	1	84	16
PA	99	1	95	5
Dissociation of *smg* p21B[d]				
Control	99	1	39	61
PI	99	1	89	11
PA	99	1	96	4

a) The binding of *smg* p21B (8 pmol) to erythrocyte ghosts (22 μg of protein) was assayed in the presence or absence of *smg* p21 GDS (48 pmol) as described.[15]
b) The amounts of *smg* p21B in the membrane and soluble fractions are expressed as percent relative to total amounts.
c) PI or PA was used for the assays in an amount of 100 μg/ml.
d) The dissociation of prebound *smg* p21B (8 pmol) from erythrocyte ghosts was assayed in the presence or absence of *smg* p21 GDS (48 pmol) as described.[15]

inhibited the *smg* p21 GDS actions to inhibit the binding of *smg* p21B to membranes and to induce the dissociation of prebound *smg* p21B from the membranes (Table II).

We have shown here that acidic phospholipids including PI, PIP, PIP_2, PA, and PS affect neither GTP-binding, GTPase, nor membrane-binding activity of *smg* p21B but inhibit the *smg* p21 GDS actions to stimulate the GDP/GTP exchange reaction, to inhibit the binding of *smg* p21B to membranes, and to induce the dissociation of prebound *smg* p21B from the membranes. *smg* p21B has a polybasic region upstream of the geranylgeranylated C-terminal region and the phosphorylation of the serine residue just downstream of this polybasic region reduces the membrane-binding activity of *smg* p21B and renders *smg* p21B sensitive to the *smg* p21 GDS actions to stimulate the GDP/GTP exchange reaction, to inhibit the binding of *smg* p21B to membranes, and to induce the dissociation of prebound *smg* p21B from the membranes.[18] On the basis of these observations, we have proposed that the acidic polar head groups of membrane phospholipids may interact with the polybasic region of *smg* p21B. The present results support the previous proposal and suggest that the acidic polar head groups of membrane phospholipids interact with the polybasic region of *smg* p21B and thereby inhibit the interaction of *smg* p21B with its GDS.

It is well known that PI, PIP, PIP_2, and PA are rapidly metabolized in respones to various extracellular signals and that the amounts of these acidic phospholipids rapidly vary.[23] It is possible that the activation of *smg* p21 by its GDS and protein kinase A-catalyzed phosphorylation may be affected by the extracellular signal-linked metabolism of these membrane phospholipids. This possibility is currently being investigated.

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