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Biophysical parameters of the Sec14 phospholipid exchange cycle – Effect of lipid packing in membranes

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Abstract

Sec14, a yeast phosphatidylinositol/phosphatidylcholine transfer protein, functions at the trans-Golgi membranes. It lacks domains involved in protein-protein or protein-lipid interactions and consists solely of the Sec14 domain; hence, the mechanism underlying Sec14 function at proper sites remains unclear. In this study, we focused on the lipid packing of membranes and evaluated its association with in vitro Sec14 lipid transfer activity. Phospholipid transfer assays using pyrene-labelled phosphatidylcholine suggested that increased membrane curvature as well as the incorporation of phosphatidylethanolamine accelerated the lipid transfer. The quantity of membrane-bound Sec14 significantly increased in these membranes, indicating that "packing defects" of the membranes promote the membrane binding and phospholipid transfer of Sec14. Increased levels of phospholipid unsaturation promoted Sec14-mediated PC transfer, but had little effect on the membrane binding of the protein. Our results demonstrate the possibility that the location and function of Sec14 are regulated by the lipid packing states produced by a translocase activity at the trans-Golgi network.

Keywords

Phosphatidylinositol; Phosphatidylcholine; Packing defects; Fluorescence; Pyrene

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Sec14, a yeast phosphatidylinositol (PI)/phosphatidylcholine (PC) transfer protein, functions as a mediator of membrane trafficking from the trans-Golgi network (TGN) [1–3]. This protein is considered to coordinate PC and PI metabolisms through its PC/PI binding ability. In PC metabolism, the PC-bound Sec14 inhibits the CDP-choline pathway [4,5] resulting in elevated diacylglycerol (DAG) levels in the trans-Golgi membranes [6,7]. DAG is a cone-shaped lipid with a small headgroup and is known to assist membrane bending [8]. Sec14 enhances PI 4-OH kinase (Pik1) activity and increases phosphatidylinositol-4-phosphate (PI4P) levels in the Golgi membranes [1,2,9], which recruits proteins that drive vesicle formation [10]. Sec14 is likely to promote the Pik1 activity through its PC/PI exchange [1,2].

The phospholipid binding domain of Sec14 consists of a hydrophobic pocket that accommodates one phospholipid molecule and an amphipathic gate helix that functions as a lid that opens/closes the pocket [11]. The three-dimensional structure of Sec14 co-crystallized with two molecules of β-octylglucoside represents an open conformation (Fig. 1D) [11], whereas that of Sfh1 (closest homolog of Sec14) co-crystallized with PI or PC is almost identical to the former, except that the phospholipid acyl chain region is covered by the gate helix [1]. Therefore, the former is considered to represent a transient conformation during the lipid exchange reaction on the membrane, while the latter corresponds to a closed conformation during lipid acquisition. Consistent with this observation, our previous study demonstrated that Sec14 present on lipid vesicles does not dissociate from the membrane until it retains the appropriate ligand [12], suggesting a functional link between phospholipid binding/exchange and membrane association.

Unlike other Sec14 superfamily members that consist of several domains (such as GOLD and PH domains among others) involved in protein-protein and protein-lipid interaction, this prototype of protein consists solely of the Sec14 domain [13]. This gives rise to the conundrum of how Sec14 recognizes Golgi membranes. In the previous study, we observed that while acidic phospholipids (such as PI and phosphatidylserine (PS)) accelerate the phospholipid exchange cycle, they do not enhance membrane binding properties of Sec14. Interestingly, however, the increase in membrane curvature promotes both membrane binding and lipid transfer of Sec14 [12]. Therefore, morphological characteristics of membranes and spontaneous curvature of membrane lipids may play a role in the localization and function of the protein. Organelle membranes each have a unique lipid composition [14,15]; for example, the plasma membranes are rich in saturated lipids, sphingolipids, and sterol, whereas the Golgi membranes primarily comprise of unsaturated lipids and have a lower sterol content (approximately 10%) [16]. Herein, we focused on the differences in the lipid packing state and evaluated how they affect the Sec14-mediated lipid transfer in vitro.

2. Material & methods

2.1. Lipids

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dio-leoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) were procured from NOF Corporation (Tokyo, Japan). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) was procured from Avanti Polar Lipids (Alabaster, AL, USA). 1-Palmitoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (py-PC) was purchased from Molecular Probes (Eugene, OR, USA). Ergosterol was acquired from Sigma-Aldrich Corporation (St. Louis, MO, USA).

2.2. Sample preparation

Large unilamellar vesicles (LUVs) and small unilamellar vesicles (SUVs) were prepared using Tris-HCl buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mg/mL NaN_3, pH 7.4) by extrusion and sonication, respectively, as described previously [12]. The mean diameters of LUVs and SUVs were measured by dynamic light scattering using an FPAR-1000 instrument (Otsuka Electronics, Osaka, Japan) and determined to be approximately 120 and 30 nm, respectively. The total phospholipid concentration of PC LUVs was determined using a choline assay kit (Fujifilm Wako Pure Chemical Corporation, Tokyo, Japan). The phospholipid concentrations of PE- or PS-containing LUVs were measured by phosphorus analysis [17].

Sec14 was expressed and purified as described previously [1,12,18]. Briefly, octahistidine-tagged wild-type Sec14 and PC-binding-defective mutant ($Sec14^{S173I,T175I}$) were grown in *Escherichia coli* BL21 (DE3; New England BioLabs Inc., Ipswich, MA). Recombinant proteins were purified with TALON metal affinity beads (Clontech, Mountain View, CA). The purified proteins were dialyzed against Tris-HCl buffer containing β-mercaptoethanol (10 mM Tris, 5 mM β-mercaptoethanol, 150 mM NaCl, 1 mM EDTA, 1 mg/mL NaN_3, pH 7.4).

2.3. Lipid transfer and extraction assays

Phospholipid transfer assays using py-PC were conducted as described previously [12,19]. Briefly, donor vesicles (LUVs or SUVs containing 16 mol% py-PC) and acceptor vesicles (lacking py-PC) were mixed and incubated at 37 °C for 10 min. The fluorescence intensity of a pyrene-monomer (378 nm) and -excimer (478 nm) was measured every 10 s for 10 min with excitation at 345 nm in an F4500 spectro-fluorometer (Hitachi High-Tech Science Corporation, Tokyo, Japan). After confirming that the fluorescence intensity is constant, the Sec14 solution was injected into the donor/acceptor mixture and the pyrene fluorescence was further monitored. The final concentrations of Sec14, donor, and acceptor were 50 μg/mL (1.4 μM), 20 μM, and 40 μM, respectively.

py-PC extraction from vesicles by Sec14 was assayed using a similar procedure, as described above, without acceptor vesicles. In both the lipid transfer and extraction assays, the pyrene-excimer/monomer intensity ratio (*E/M*) was calculated from the fluorescence

intensities and normalized to the *E/M* value recorded immediately prior to the Sec14 injection.

py-PC delivery from Sec14 to acceptor vesicles was evaluated using a modified version of the phospholipid transfer assay. Sec14 was incubated with donor vesicles consisting of py-PC/POPC (16:84) at 37 °C for 30 min. A suspension of acceptor vesicles was injected into the mixture 10 min after initiation of the pyrene fluorescence measurement. The *E/M* was normalized to the value prior to the acceptor injection.

2.4. Membrane binding assays

To evaluate the binding of Sec14 to the phospholipid membranes, vesicle floatation assays were conducted as described previously [12]. Briefly, Sec14 (175 μg/mL = 5 μM) was incubated with LUVs (2 mM total lipids) in Tris-HCl buffer at 37 °C for 30 min, and the mixture was separated into membrane-free (bottom) and membrane-bound (top) fractions using sucrose density gradient ultracentrifugation. Sec14 in each fraction was analyzed using SDS-PAGE and Coomassie brilliant blue staining. The statistical significance of differences in the binding concentration between mean values was analyzed using the unpaired *t*-test.

3. Results

3.1. Effect of membrane curvature on Sec14 activity

In the previous study, we succeeded in detecting the Sec14-mediated phospholipid transfer using small-angle neutron scattering, which demonstrated more rapid lipid transfer between SUVs (high curvature membranes) than that between LUVs (low curvature membranes) [12]. Fluorescence-based phospholipid transfer assays were conducted to confirm Sec14's binding propensity to high curvature membranes. In this method, the transfer of py-PC from donor to acceptor vesicles can be detected as a reduction in the *E/M* values. As shown in Fig. 1A, the *E/M* values gradually decreased when Sec14 was added to the mixture of donor LUV (py-PC/POPC = 16:84) and acceptor LUV (POPC 100%), and the decay was accelerated when SUVs, and not LUVs, were used as the donor and acceptor. The curvature-sensitive lipid transfer was supported by the observation that a pair of donor LUV and acceptor SUV induced a negligible reduction of the *E/M* values. This implies that Sec14 binds exclusively to acceptor SUVs and thus cannot extract py-PC from donor LUVs. When a donor SUV and acceptor LUV pair was used, there was a modest reduction in the *E/M* values. However, the *E/M* value is reduced even when Sec14 only extracts py-PC from donor vesicles. Indeed, the decay profile of the donor SUV and acceptor LUV pair was almost identical to that in the absence of acceptor LUV (Fig. 1B). This indicates that Sec14 exchanges lipid preferentially between curved membranes. Next, we evaluated lipid delivery to acceptor vesicles. In this assay, Sec14 was initially incubated with donor LUVs (py-PC/POPC = 16:84), followed by the injection of acceptor vesicles (POPC LUVs or SUVs) into the mixture. Since the *E/M* values prior to the acceptor injection were stable, it was inferred that Sec14-mediated py-PC extraction from donor vesicles had attained a steady state. The addition of acceptor LUVs reduced the *E/M* values (Fig. 1C), suggesting the delivery of py-PC to acceptor LUVs. The addition of acceptor SUVs resulted in more rapid reduction of the *E/M* values, suggesting the promotion of py-PC delivery. These data indicate that Sec14

exhibits greater affinity for membranes with higher curvature, which is consistent with a previous result that suggested that the quantity of Sec14 binding to SUVs is greater than that binding to LUVs [12]. Increased membrane curvature is known to create gaps between lipid-headgroups and enhance the binding of amphipathic helices to membranes [20–23]. We suggest that the amphipathic gate helix of Sec14 (Fig. 1D) is associated with the enhancement of curvature-dependent binding.

3.2. Effect of cone-shaped lipids

Along with a high membrane curvature, cone-shaped lipids with a small headgroup result in lipid packing defects as well [24]. To examine their effect on the lipid transfer activity of Sec14, we used POPE as a cone-shaped lipid. As shown in Fig. 2A, incorporation of 30 mol % POPE (py-PC/POPC/POPE = 16:54:30 and POPC/POPE = 70:30 for donor and acceptor vesicles, respectively) brought about faster Sec14-mediated py-PC transfer compared to that induced by POPC LUVs. Additionally, we evaluated the py-PC extraction by Sec14 from donor vesicles and the py-PC delivery to acceptor vesicles. In the extraction assay (Fig. 2B), Sec14 reduced the *E/M* to lower values when the donor LUVs contained 30 mol% POPE, indicating that Sec14 extracts more py-PC molecules from POPE-containing membranes than from POPE-free membranes. Presumably, this is because substitution of POPC with POPE, which is not a ligand for Sec14, reduces the number of PC molecules that compete with py-PC. Here, POPE is unlikely to enhance the extraction rate, based on observations from the initial decays. In the delivery assay, either of the two acceptor LUVs (POPC 100% or POPC/POPE = 70:30) was mixed with Sec14 preincubated with the same donor LUVs (py-PC/POPC = 16:84). As a result, the py-PC delivery to the POPE-containing vesicles was more prominent compared to that to POPE-free vesicles (Fig. 2C). These results indicate that POPE promotes the Sec14 lipid transfer, particularly the lipid delivery process.

Next, we examined whether POPE enhances the membrane binding of Sec14. The membrane binding assay using POPC LUV and POPC/POPE (50:50) LUV revealed that relatively small quantities (approximately 13%) of Sec14 were recovered from the membrane-bound fraction; however, the binding quantity did not differ between these two vesicles (Fig. 3A). Previously, we reported that Sec14 is prone to dissociate from the membrane as the protein acquires a ligand such as PC or PI [12]. In the binding assay, since both types of vesicles contained POPC, gaining of POPC might stimulate Sec14 to dissociate from the membrane, such that the effect exerted by POPE on the binding is less pronounced. To circumvent this limitation, we further conducted binding experiments in two ways. First, the effect of POPE on Sec14 binding was evaluated using ligand-free vesicles by replacing POPC by POPS. As expected, Sec14 exhibited increased binding (approximately 34%) to POPS LUVs compared to POPC LUVs, and more importantly, approximately 2.3-fold of Sec14 (77%) bound to POPS/POPE (50:50) LUVs (Fig. 3B). However, replacement of POPC with the acidic lipid POPS can markedly alter the electrostatic interactions between membranes and proteins. Therefore, we next examined the binding using POPC-containing vesicles and a Sec14 mutant that is inactive in POPC extraction. $Sec14^{S173I,T175I}$ is a mutant lacking PC binding/transfer ability (but having PI binding/transfer ability) [1,12]. Only about 8% of $Sec14^{S173I,T175I}$ bound to POPC LUVs, and the incorporation of 50% POPE significantly enhanced the binding of the mutant (48%, Fig. 3C). These results suggest that

the cone-shaped lipid, POPE, promotes the membrane binding of Sec14 and that the protein promptly dissociates from the membrane when it acquires a ligand.

3.3. Effect of sterol

The sterol content is known to vary between organelles. For example, plasma membranes contain 30–40% and Golgi membranes have a lower sterol content (approximately 10%) [14,16]. Since yeasts contain ergosterol, we examined its effects on the Sec14 lipid transfer. It has been reported that POPC/ergosterol (70:30) membrane exhibits phase separation into ergosterol-rich L_o and ergosterol-poor L_d phases at 37 °C [25]. Fluorescence spectra of py-PC indicated that the *E/M* value of py-PC/POPC/ergosterol (16:54:30) LUV was approximately 1.5-fold higher than that of py-PC/POPC (16:84) LUV (data not shown). This is presumably because py-PC molecules are excluded from the L_o phase and predominantly localize in the L_d phase, resulting in the increased local py-PC concentration [26,27]. This makes it challenging to compare the lipid transfer among vesicles with different sterol concentrations. Therefore, we conducted the lipid delivery assay, where acceptor vesicles with different ergosterol concentrations were injected to the preincubated mixture of Sec14 and py-PC/POPC (16:84) LUVs. As a result, the *E/M* decay profiles were not altered by the presence of 10 or 30 mol% ergosterol (Fig. 4), indicating that ergosterol exerts limited effects on Sec14-mediated PC lipid transfer.

3.4. Effect of phospholipid acyl chain unsaturation

Golgi membranes are primarily composed of unsaturated phospholipids [15,16]. These lipids occupy a larger molecular area than saturated lipids and induce lipid packing defects in membranes [28,29]. Therefore, we expected that apart from the membrane curvature and the cone-shaped lipids, the level of phospholipid unsaturation would modify the membrane binding and lipid transfer activity of Sec14 as well. Here, DPPC and DOPC were used as saturated and unsaturated lipids, respectively, and their effect on Sec14 lipid transfer was evaluated using LUVs consisting of POPC and these lipids. The Sec14-mediated py-PC transfer between LUVs containing 30 mol% DOPC (py-PC/POPC/DOPC = 16:54:30 and POPC/DOPC = 70:30 for donor and acceptor, respectively) was accelerated as compared to that between POPC LUVs (py-PC/POPC = 16:84 and POPC 100%, respectively) (Fig. 5A). Conversely, inclusion of 30 mol% DPPC (py-PC/POPC/DPPC = 16:54:30 and POPC/DPPC = 70:30, respectively) induced the opposite effect (Fig. 5A). Here, it should be noted that POPC/DPPC (70:30) membranes do not exhibit phase separation and remain in the liquid-crystalline (L_α) phase at the experimental temperature (37 °C) [30]. The results support the proposition that the increased levels of phospholipid unsaturation promote Sec14-mediated PC transfer.

To further investigate the effect of acyl chain unsaturation, py-PC extraction (to Sec14) and delivery (to acceptor vesicles) were analyzed separately. Notably, py-PC extraction was unaffected by both DOPC and DPPC (Fig. 5B). This indicates that changes in the unsaturation levels of membranes, as well as the POPE incorporation, do not alter the py-PC extraction rate, and the extent to which PCs compete for py-PC extraction does not differ significantly between POPC, DOPC, and DPPC. Conversely, py-PC delivery from Sec14 (preincubated with py-PC/POPC (16:84) LUVs) to acceptor vesicles was accelerated and

decelerated when the acceptor vesicles contained 30 mol% DOPC and DPPC, respectively (Fig. 5C). Therefore, the unsaturation levels were observed to alter only the lipid delivery process of Sec14.

We next evaluated the effect of the unsaturation levels on the membrane binding of Sec14. Since wild-type Sec14 tends to dissociate from the PC-containing membranes as described above, the PC-binding-defective mutant $Sec14^{S173I,T175I}$ was used instead. As shown in Fig. 6, the quantity of $Sec14^{S173I,T175I}$ binding to POPC LUVs, POPC/DPPC (50:50) LUVs, and POPC/DOPC (50:50) LUVs was comparable within the experimental error. This result suggests that the change in the degree of unsaturation of the membranes does not significantly modulate the binding of Sec14. Therefore, enhanced PC delivery to the membranes rich in the unsaturated lipids may be due to factors other than the membrane binding of Sec14 (see Discussion).

4. Discussion

In this study, we conducted the phospholipid transfer assays using py-PC. Real-time monitoring of pyrene monomer/excimer fluorescence enabled the detection of the lipid transfer dynamics in a time-scale within several 10 min, which was hardly possible by the method using radioisotope-labelled lipids. MD simulation has shown that the pyrene moiety of py-PC only perturbs the bilayer locally within a distance of 1.5 nm from pyrene and the average molecular area is not significantly changed compared to a pure DPPC membrane [31]. Previously, we reported the transport of deuterium-labelled POPC by Sec14 using neutron scattering [12], and the effect of the membrane curvature is consistent with the present result obtained with py-PC. This supports the validity of the method using py-PC.

Yeast Sec14 mediates PC and PI exchange in the Golgi membrane and promotes membrane budding in the TGN [1,2]. Cell fractionation revealed that Sec14 is localized in the cytosol and microsomes [32,33], and immunofluorescence showed that Sec14 apparently colocalizes with the TGN marker Kex2 [32], suggesting that the TGN is the major membrane interaction site of Sec14. Sec14 is considered to stimulate PI 4-OH kinase activity by executing multiple rounds of phospholipid exchange in the Golgi membrane [34]. However, the mechanism underlying Golgi membrane recognition by Sec14 remains unclear. We previously demonstrated that acidic phospholipids (PI and PS) enhanced Sec14-mediated PC transfer activity by 2.7–3.9 fold, whereas they do not modify Sec14 membrane binding [12], suggesting that these lipids are not involved in the localization of Sec14 to the Golgi membranes. In this study, we revealed that “packing defects” of the membranes promote the membrane binding of Sec14, and thereby accelerate lipid transfer.

Our fluorescence-based lipid transfer assays revealed that while incubation of Sec14 with the mixture of donor LUV and acceptor SUV reduced the *E/M* value negligibly, mixing the acceptor SUV with the protein that was preincubated with donor LUV rapidly reduced the *E/M* value. This demonstrates higher propensity of Sec14 to deliver its bound lipid to membranes with higher curvature. Sec14-like 3, a mammalian Sec14 homolog protein, was reported to exhibit high affinity for membranes with high curvature [35] as well. In addition to the membrane curvature, incorporation of POPE was found to bring about increased

membrane binding of Sec14. Drs2, a Golgi-localized P4-AT-Pase in *Saccharomyces cerevisiae* functions as a phospholipid translocase. Translocation of NBD-labelled-PS and to a lesser extent -PE from the luminal leaflet to the cytosolic leaflet has been observed with purified TGN membranes containing wild-type Drs2 [36]. The translocase activity of the P4-ATPase is considered to induce an imbalance in the level of lipids between the leaflets, generate local membrane bending, and drive membrane curvature [37]. Indeed, this activity is essential for recruitment of the Arf GTPase-activating protein (ArfGAP) Gcs1, which contains an amphipathic helical motif, from cytosol to TGN [38]. We presume that Sec14 also senses the membrane curvature and packing defects via the amphipathic gate helix (Fig. 1D) to localize in the TGN. Notably, PI4P, which is generated by the Sec14-mediated activation of Pik1, recruits adaptor proteins that mediate clathrin-dependent trafficking from the TGN [39]. The mechanism underlying coordination of Sec14-mediated membrane trafficking by membrane curvature is an interesting topic that needs to be addressed in future research.

Unsaturated and saturated lipids promote and suppress the phospholipid delivery of Sec14 to acceptor membranes, respectively. These lipids, however, had only a marginal effect on Sec14 binding (Fig. 6). These results imply that the regulation of the Sec14 lipid transfer activity by phospholipid unsaturation levels is not attributed to the change in the quantity of Sec14 binding to membranes. Saturated lipids promote tighter membrane packing and make lipid insertion into membranes more difficult. The plasma membranes are abundant in saturated lipids [14,15] and the Golgi membranes are primarily composed of unsaturated lipids [15,28], which might be a factor that causes Sec14 to function in the latter organelle.

5. Conclusions

In the present study, we evaluated the effect of membrane curvature and lipid unsaturation levels on the Sec14 lipid transfer activity. We revealed that the lipid packing defects promote the membrane binding and phospholipid transfer of Sec14. Increased membrane curvature and incorporation of cone-shaped lipids reduce the ratio of headgroup size/cross-sectional molecular area, which increases the surface exposure of the hydrophobic core of the lipid bilayer [20–23,40]. This promotes protein binding to negate unfavorable hydrophobic hydration. Additionally, membranes abundant in unsaturated lipids could help insert the phospholipid residues retained by Sec14 into the membranes. As the P4-ATPase in the TGN increases the number of aminophospholipids in the cytosolic leaflet and the local membrane curvature in the TGN, Sec14 may possibly recognize the region, exchange lipids, and promote vesicle formation.

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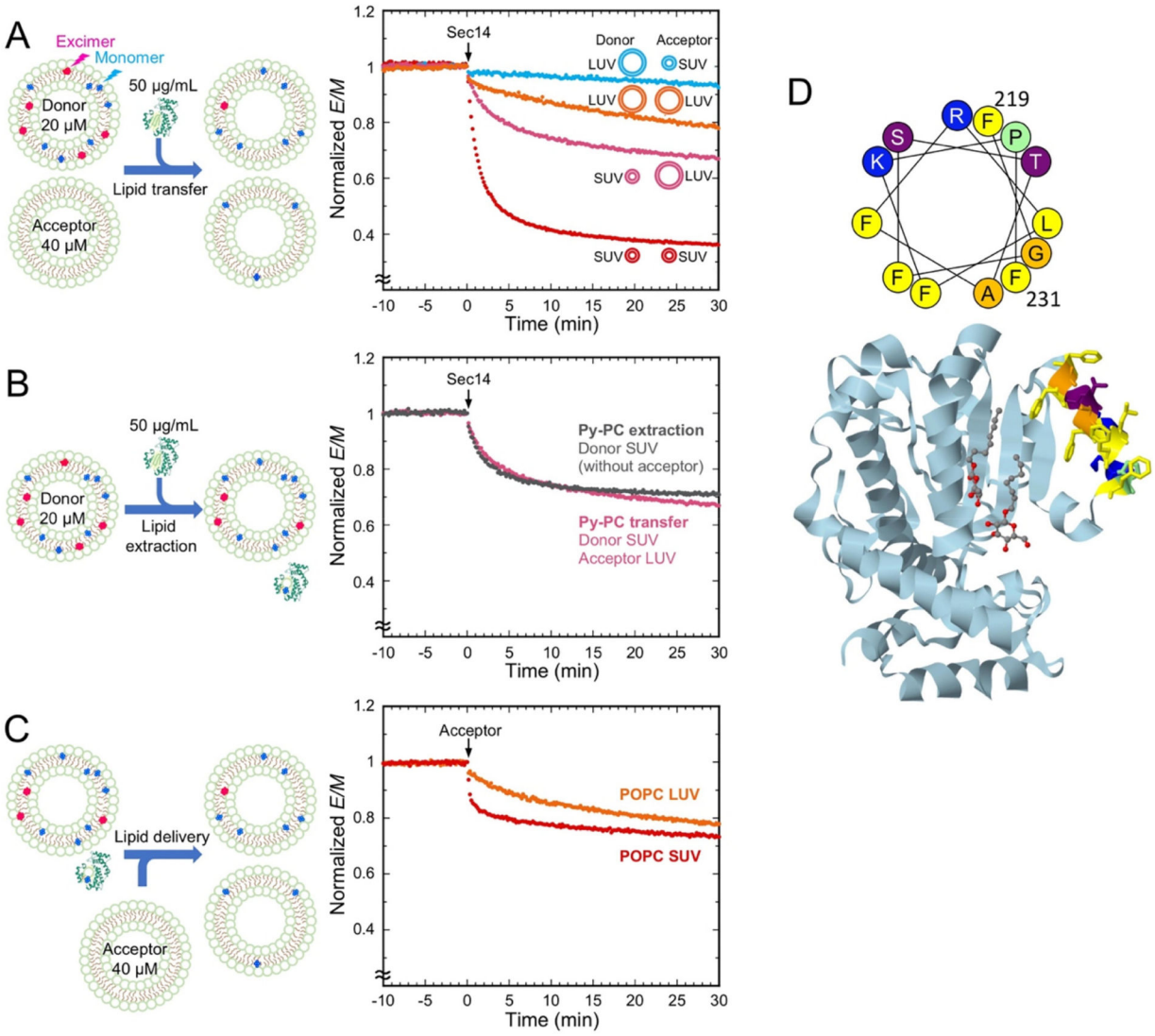

Fig. 1.

Effect of membrane curvature on the PC transfer activity of Sec14. **(A)** Lipid transfer assays were conducted by injecting Sec14 (50 μg/mL) into mixtures of donor vesicles (LUVs or SUVs) containing 16 mol% py-PC (py-PC/POPC = 16:84, 20 μM lipids) and acceptor vesicles (POPC LUVs or SUVs, 40 μM lipids). **(B)** Lipid extraction assays were conducted by injecting Sec14 (50 μg/mL) into donor SUVs (py-PC/POPC = 16:84, 20 μM, gray). Data in magenta represent the result of the lipid transfer assay shown in (A) using donor SUV and acceptor LUV pair. **(C)** Lipid delivery assays were conducted by injecting acceptor vesicles (POPC LUVs or SUVs) into the preincubated mixture of Sec14 and donor LUVs (py-PC/POPC = 16:84). The final concentrations of Sec14 and lipids were the same as those indicated in (A). **(D)** Helical-wheel representation of the 219–231 segment (amphipathic gate helix) and crystal structure of Sec14 (PDB ID: 1AUA) highlighted the gate helix. Yellow, Leucine and phenylalanine; orange, glycine and alanine; purple, serine and threonine; blue, lysine and arginine. Ligands (two β-octylglucoside molecules) are shown by a ball-and-stick model.

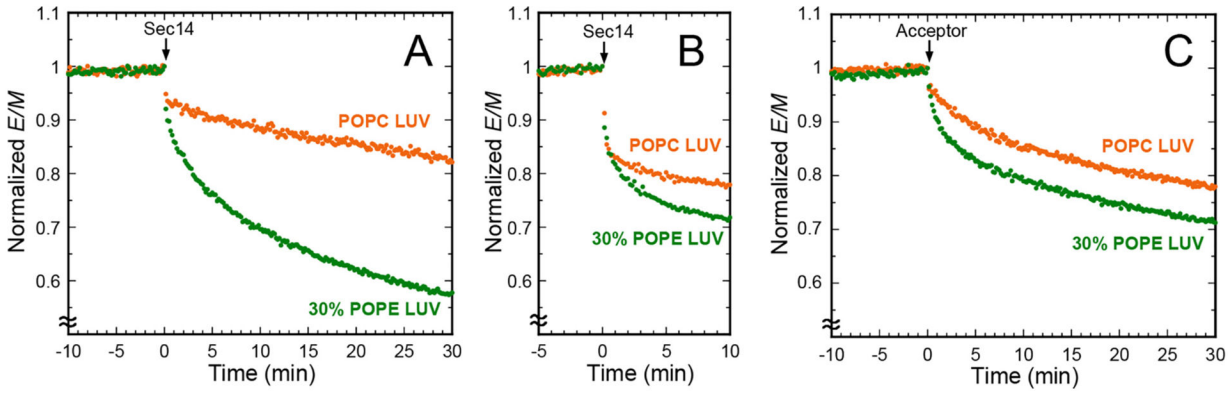

Fig. 2.

Effect of a cone-shaped lipid on the PC transfer activity of Sec14. **(A)** Lipid transfer assays were conducted by injecting Sec14 into mixtures of donor and acceptor LUVs. Donor and acceptor compositions used were [py-PC/POPC (16:84) and POPC 100%] or [py-PC/POPC/POPE (16:54:30) and POPC/POPE (70:30)], respectively. **(B)** Lipid extraction assays were conducted by injecting Sec14 into donor LUVs (py-PC/POPC = 16:84 or py-PC/POPC/POPE = 16:54:30). **(C)** Lipid delivery assays were conducted by injecting acceptor LUVs (POPC 100% or POPC/POPE = 70:30) into the preincubated mixture of Sec14 and donor LUVs (py-PC/POPC = 16:84). Note that the same batches of protein and donor vesicles as Fig. 1(C) were used in these assays and hence the same trace is used for the control data (with POPC LUV as acceptor vesicles). The final concentrations of Sec14 and lipids were the same as those indicated in Fig. 1.

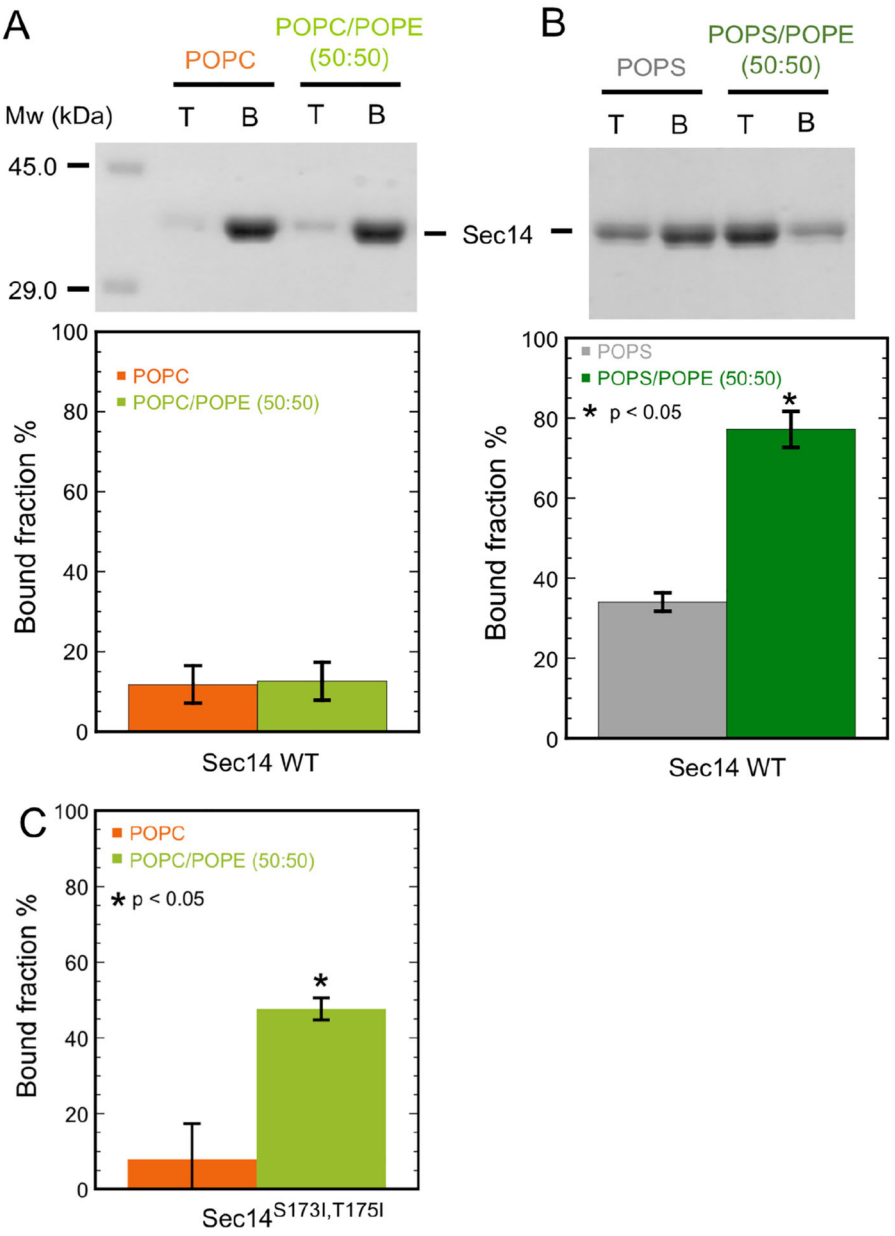

Fig. 3.

Effect of a cone-shaped lipid on the membrane binding of Sec14. Vesicle floatation assays were conducted by incubating wild-type Sec14 (175 μg/mL) with 2 mM POPC 100% or POPC/POPE (50:50) LUVs **(A)**, or with POPS 100% or POPS/POPE (50:50) LUVs **(B)**, or by incubating $Sec14^{S173I,T175I}$ (175 μg/mL) with 2 mM POPC 100% or POPC/POPE (50:50) LUVs **(C)**. The mixture was separated by ultracentrifugation into top (T) and bottom (B) fractions and Sec14 from each fraction was analyzed by SDS-PAGE as shown in the upper panel in A and B (the SDS-PAGE image for $Sec14^{S173I,T175I}$ is shown in Fig. 6). Each bar in the graph represents the percentage of lipid-bound fraction against total Sec14 content (that is, top fraction/(top fraction + bottom fraction)). The bars represent the mean ± S.D. from four (A and B) or three (C) independent experiments.

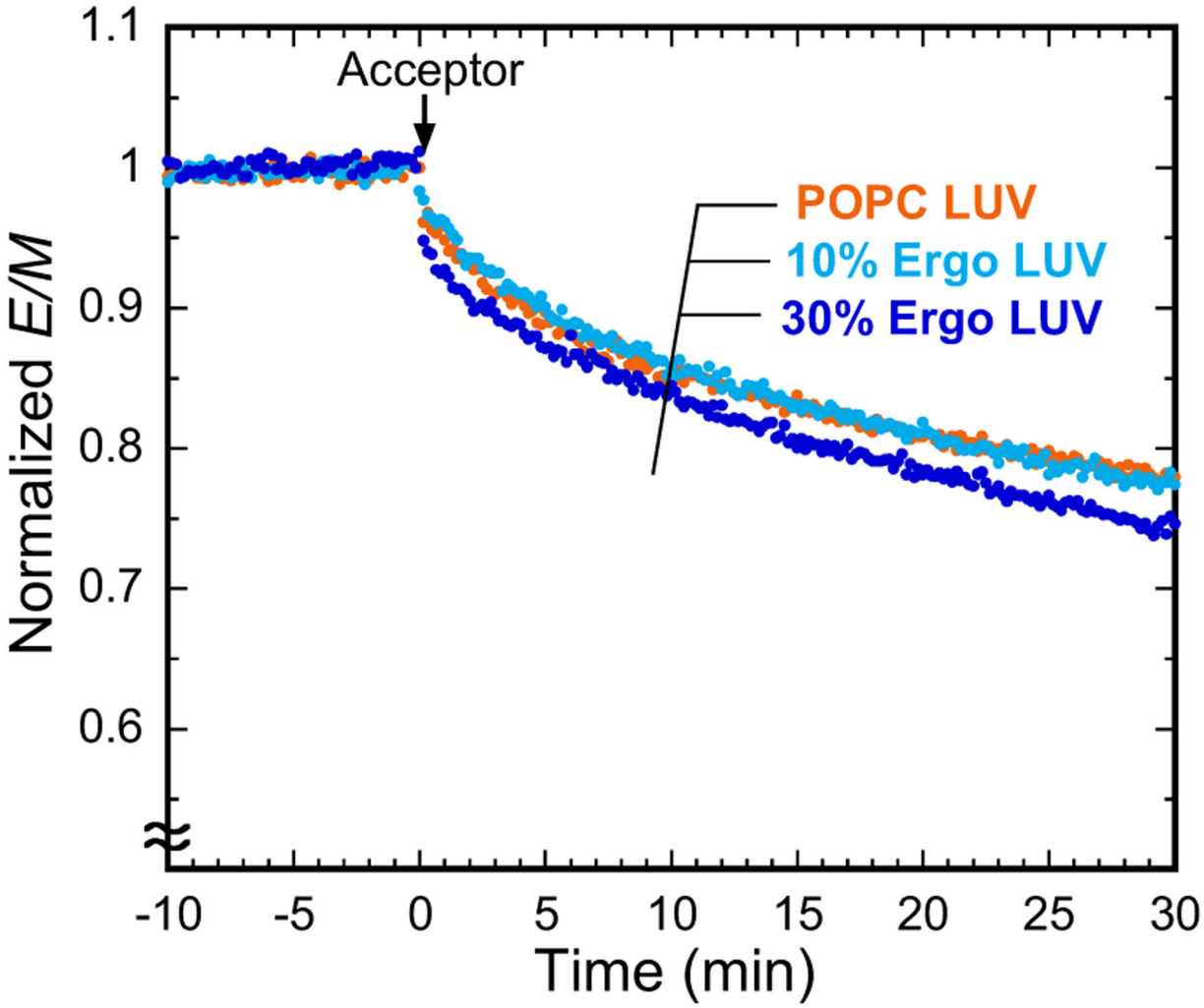

Fig. 4.

Effect of sterol on Sec14 PC transfer activity. Lipid delivery assays were conducted by injecting acceptor LUVs (POPC 100%, POPC/ergosterol = 90:10 or 70:30) into the preincubated mixture of Sec14 and donor LUVs (py-PC/POPC = 16:84). Note that the same batches of protein and donor vesicles as Fig. 1(C) were used in these assays and hence the same trace is used for the control data (with POPC LUV as acceptor vesicles). The final concentrations of Sec14 and lipids were the same as those indicated in Fig. 1.

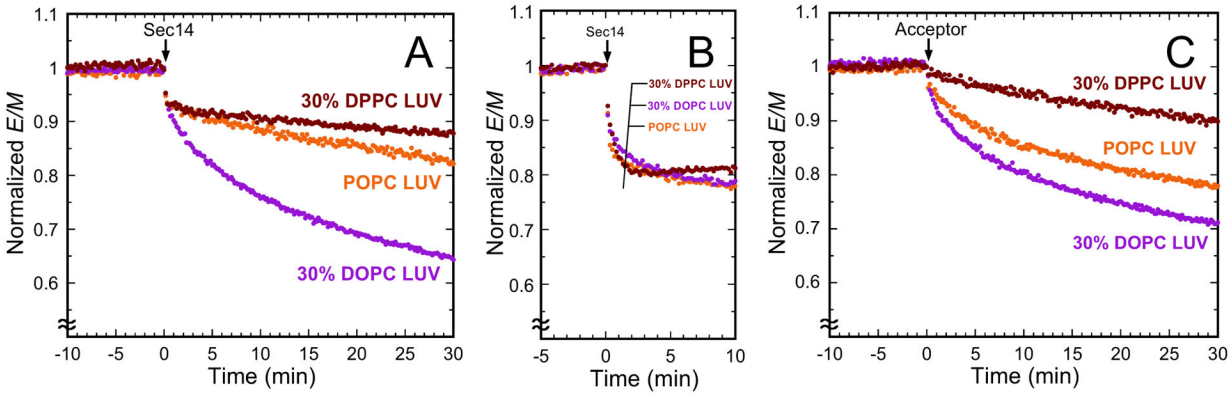

Fig. 5.

Effect of phospholipid acyl chain unsaturation on the PC transfer activity of Sec14. **(A)** Lipid transfer assays were conducted by injecting Sec14 into mixtures of donor and acceptor LUVs. Donor and acceptor compositions were [py-PC/POPC (16:84) and POPC 100%], [py-PC/POPC/DPPC (16:54:30) and POPC/DPPC (70:30)], or [py-PC/POPC/DOPC (16:54:30) and POPC/DOPC (70:30)], respectively. **(B)** Lipid extraction assays were conducted by injecting Sec14 into donor LUVs (py-PC/POPC = 16:84, py-PC/POPC/DPPC = 16:54:30, or py-PC/POPC/DOPC = 16:54:30). **(C)** Lipid delivery assays were conducted by injecting acceptor LUVs (POPC 100%, POPC/DPPC = 70:30, or POPC/DOPC = 70:30) into the preincubated mixture of Sec14 and donor LUVs (py-PC/POPC = 16:84). Note that the same batches of protein and donor vesicles as Fig. 1(C) were used in these assays and hence the same trace is used for the control data (with POPC LUV as acceptor vesicles). The final concentrations of Sec14 and lipids were the same as those indicated in Fig. 1.

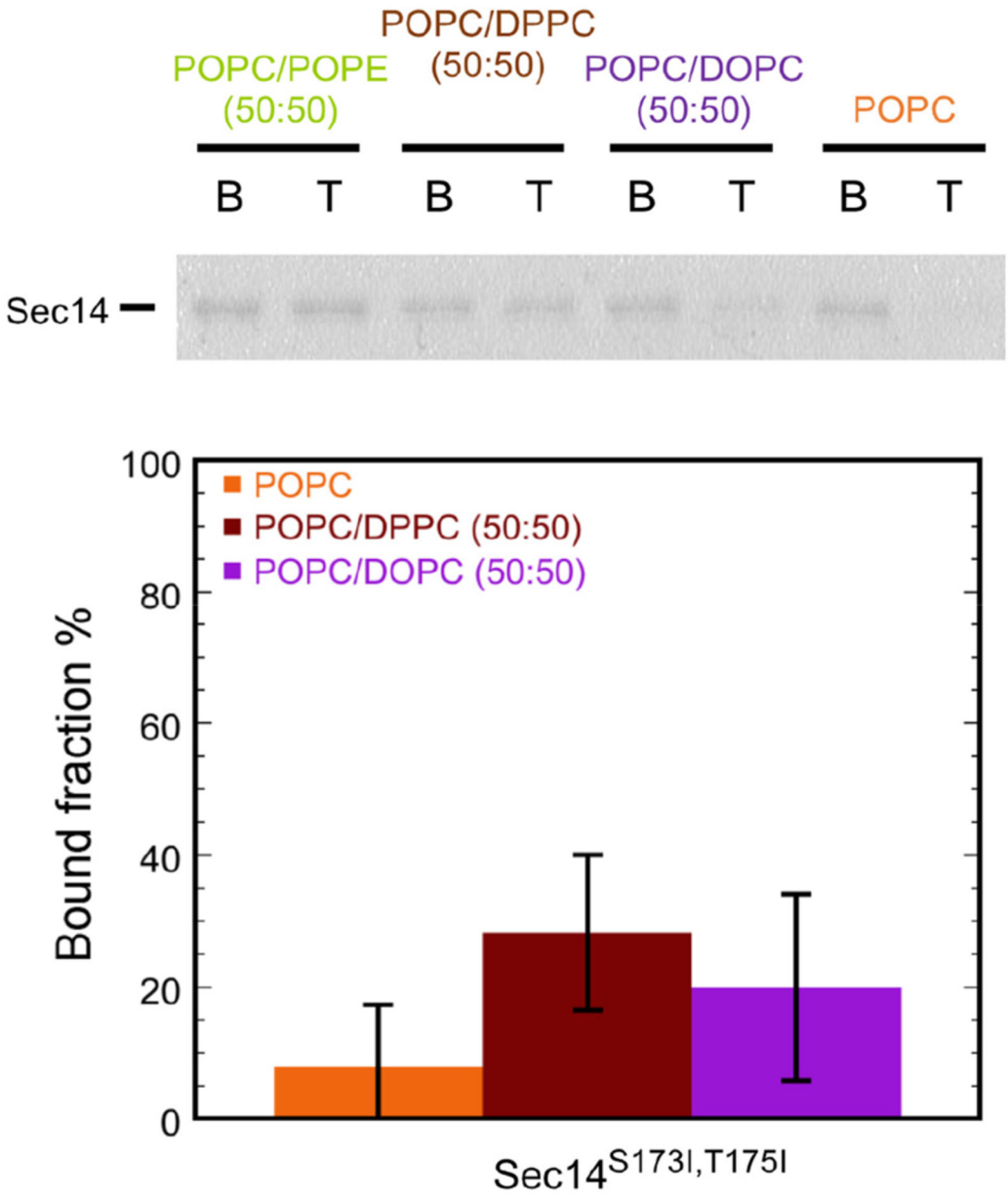

Fig. 6.

Effect of phospholipid acyl chain unsaturation on the membrane binding of Sec14. Vesicle floatation assays were conducted by incubating $Sec14^{S173I,T175I}$ (175 μg/mL) with POPC 100%, POPC/DPPC (50:50), or POPC/DOPC (50:50) LUVs (2 mM lipids) and separating into top (T) and bottom (B) fractions. Sec14 of each fraction was analyzed by SDS-PAGE as shown in the upper panel, where data for POPC/POPE (50:50) are also displayed (see Fig. 3). Each bar in the lower panel represents percentage of lipid-bound fraction against total Sec14 quantity (that is, top fraction/(top fraction + bottom fraction)). Bars indicate the mean ± S.D. from three independent experiments.