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The tail domain of tomosyn controls membrane fusion through tomosyn displacement by VAMP2

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ABSTRACT

Neurotransmitter release is regulated by SNARE complex-mediated synaptic vesicle fusion. Tomosyn sequesters target SNAREs (t-SNAREs) through its C-terminal VAMP-like domain (VLD). Cumulative biochemical results suggest that the tomosyn–SNARE complex is so tight that VAMP2 cannot displace tomosyn. Based on these results, the tomosyn–SNARE complex has been believed to be a dead-end complex to inhibit neurotransmitter release. On the other hand, some studies using siRNA depletion of tomosyn suggest that tomosyn positively regulates exocytosis. Therefore, it is still controversial whether tomosyn is a simple inhibitor for neurotransmitter release. We recently reported that the inhibitory activity of tomosyn is regulated by the tail domain binding to the VLD. In this study, we employed the liposome fusion assay in order to further understand modes of action of tomosyn in detail. The tail domain unexpectedly had no effect on binding of the VLD to t-SNARE-bearing liposomes. Nonetheless, the tail domain decreased the inhibitory activity of the VLD on the SNARE complex-mediated liposome fusion. These results indicate that the tail domain controls membrane fusion through tomosyn displacement by VAMP2. Deletion of the tail domain-binding region in the VLD retained the binding to t-SNAREs and promoted the liposome fusion. Together, we propose here a novel mechanism of tomosyn that controls synaptic vesicle fusion positively by serving as a placeholder for VAMP2.

1. Introduction

Synaptic vesicles are transported to the presynaptic plasma membrane where Ca^{2+} channels are located. Depolarization induces Ca^{2+} influx into the cytosol of nerve terminals through the Ca^{2+} channels, and this Ca^{2+} influx initiates the fusion of the vesicles with the plasma membrane, finally leading to exocytosis of neurotransmitters [1]. Soluble N-ethylmaleimide-sensitive fusion protein (NSF) attachment protein (SNAP) receptors (SNAREs) are essential for the synaptic vesicle exocytosis [2–5]. Synaptic vesicles are endowed with vesicle-associated membrane protein 2 (VAMP2) as a vesicular SNARE (v-SNARE), whereas the presynaptic plasma membrane is endowed with syntaxin-1 and SNAP-25 as target SNAREs (t-SNAREs). VAMP2 interacts with SNAP-25 and syntaxin-1 to form a stable SNARE complex [6–9]. The SNARE complex formation then brings synaptic vesicles and the plasma membrane into close apposition, and provides the energy that drives the mixing of the two lipid bilayers [3–5,9]. Although the previous study using the SNARE complex reconstituted on liposomes demonstrated that the SNARE complex was sufficient for membrane fusion, the fusion kinetics was very slow [3]. Syntaxin-1 and SNAP-25 have been shown to predominantly form a stable complex at 2:1 stoichiometry *in vitro*, where the second syntaxin-1 occupies the position of VAMP2, leading to slow down of the membrane fusion rate [10,11]. These facts indicate *in vivo* existence of a special mechanism(s) to regulate SNARE assembly for fast membrane fusion characteristic of neurotransmitter release. Some regulators of the SNARE complex formation including complexin, Munc13, Munc18, and synaptotagmin-1 were recently reported to stabilize the 1:1 syntaxin-1/SNAP-25 complex *in vitro* [12]. However, it still remains unclear how the 2:1 syntaxin-1/SNAP-25 complex formation is prevented on the presynaptic membrane.

Tomosyn, a syntaxin-1-binding protein that we originally identified [13], is one of the regulators of the SNARE complex formation [14]. Tomosyn contains N-terminal WD40 repeats, a tail domain, and a C-terminal domain homologous to VAMP2. The C-terminal VAMP-like domain (VLD) of tomosyn acts as a SNARE domain that competes with VAMP2. The structural study of the VLD revealed that the VLD, syntaxin-1, and SNAP-25 assembles into a SNARE complex-like structure (referred to as tomosyn–SNARE complex hereafter) [15]. We recently reported that the inhibitory activity of the VLD is structurally regulated by the reciprocal intramolecular bindings of its tail domain to the N-terminal WD40 repeats and

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the VLD [16]. The binding of the tail domain to the WD40 repeats exposes the VLD, leading to potent inhibition of the SNARE complex formation, whereas the binding of the tail domain to the VLD decreases its inhibitory activity on the SNARE complex formation. *In vitro*, the binding of the VLD to t-SNAREs is so tight that VAMP2 cannot displace the VLD [15]. Therefore, the tomosyn–SNARE complex has been believed to be a dead-end complex to inhibit the SNARE complex formation. Consistent with this notion, evidence is accumulating that tomosyn acts as a negative regulator for neurotransmitter release. In *Caenorhabditis elegans*, TOM-1, an orthologue of tomosyn, inhibits neurotransmitter release by modulating the priming of synaptic vesicles with UNC-13 [17,18]. Tomosyn also inhibits the exocytosis of dense core granules in adrenal chromaffin cells and PC12 cells [19,20]. Moreover, we recently reported that genetic ablation of tomosyn in mice enhances efficacy of the synaptic transmission in hippocampi [21]. By contrast, several lines of evidence suggest that tomosyn positively regulates exocytosis. Tomosyn depletion by siRNA inhibits acetylcholine release from superior cervical ganglion neurons [22] and insulin secretion from insulin-secreting INS-1E cells [23]. So far, it remains unclear how tomosyn promotes vesicle fusion.

In this study, we employed the liposome fusion assay to further understand modes of action of tomosyn on the SNARE complex-mediated membrane fusion. While the tail domain decreased the inhibitory activity of the VLD on the SNARE-mediated liposome fusion, it unexpectedly had no effect on the VLD binding to t-SNAREs. Deletion of the tail domain-binding region in the VLD promoted the liposome fusion. Together, we propose a novel mechanism of tomosyn that controls synaptic vesicle fusion positively by the tail domain binding.

2. Materials and methods

2.1. Expression and purification of recombinant proteins

The MBP-fused full-length tomosyn-1 (1–1116 aa) (MBP-tomosyn) was expressed in Sf21 cells and purified as previously described [21]. The MBP-fused fragment encompassing both the tail domain and the VLD (933–1116 aa) (MBP-Tail-VLD), and the MBP-fused fragment encompassing the VLD (1031–1116 aa) (MBP-VLD) were expressed in *Escherichia coli* and purified as previously described [16,21]. For expression of an MBP-fused fragment encompassing N-terminally truncated VLD (1075–1116 aa) (MBP-ΔN-VLD), a cDNA encoding ΔN-VLD was subcloned into the pMAL-C2 vector (New England Biolabs Inc.). MBP-ΔN-VLD was expressed in *E. coli* and purified with amylose resin in accordance with the manufacture's manual. GST-VLD and GST-ΔN-VLD were expressed in *E. coli* and purified with glutathione Sepharose. The hexa histidine-tagged tail domain (933–1050 aa) (His-Tail) was expressed in *E. coli* and purified as previously described [16]. Full-length syntaxin-1 (1–289 aa), full-length SNAP-25 (1–206 aa), and full-length VAMP2 (1–116 aa) were expressed in *E. coli* as GST-fusion proteins and purified as previously described [16,21]. To cut off the GST tags, the GST-fusion proteins were digested with PreScission protease (GE healthcare) or thrombin, and then further purified as previously described [16,21].

2.2. Liposome preparation

All lipids were purchased from Avanti Polar Lipids, and the liposomes were prepared as reported previously with slight modification [3]. Briefly, for preparation of the t-SNARE vesicles and the control vesicles, 150 μl of 10 mM lipid mixture composed of 85 mol% POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and 15 mol% DOPS (1,2-dioleoyl-sn-glycero-3-phospho-L-serine) dissolved in chloroform was dried down in test tubes by a gentle stream of N_2 gas, and any remaining traces of chloroform were then removed under vacuum for more than 30 min. The lipid films were dissolved in 500 μl of liposome buffer A (50 mM Tris/HCl, pH 8.0, 100 mM KCl, 10% (w/v) glycerol, 1 mM dithiothreitol (DTT), and 0.8% (w/v) *n*-octyl-β-D-glucopyranoside (OG)) containing 2 mg/ml syntaxin-1 and 2 mg/ml SNAP-25 for the t-SNARE vesicles and 500 μl of liposome buffer A for the control vesicles by gentle agitation for 15 min at room temperature (RT). For preparation of the fluorescently labeled v-SNARE vesicles, 100 μl of 3 mM lipid mixture composed of 82 mol% POPC, 15 mol% DOPS, 1.5 mol% NBD-DPPE (*N*-(7-nitro-2,1,3-benzzoxadiazole-4-yl)-1,2-dipalmitoyl-sn-glycero-3-phophoethanolamine), and 1.5 mol% rhodamine-DPPE (*N*-(lissamine rhodamine B sulfonyl) 1,2-dipalmitoyl-sn-glycero-3-phophoethanolamine) dissolved in chloroform was dried, and the lipid film was dissolved in 100 μl of liposome buffer B (50 mM Tris/HCl, pH 8.0, 100 mM KCl, 10% (w/v) glycerol, 1 mM DTT, and 1% (w/v) OG) containing 4.5 mg/ml VAMP2. The samples were diluted 3-fold with reconstitution buffer (25 mM HEPES/KOH pH7.4, 100 mM KCl, 10% (w/v) glycerol, and 1 mM DTT), followed by dialysis against 4 L of reconstitution buffer supplemented with 4 g of Biobeads SM-2 (Bio-Rad) using 7 kDa cutoff membranes. The dialysates were mixed with equal volumes of 80% (w/v) Nycodenz (Sigma) dissolved in reconstitution buffer. For the t-SNARE vesicles and the control vesicles, 1.3 ml of the sample was overlaid with 1 ml of 30% (w/v) Nycodenz dissolved in reconstitution buffer followed by 1 ml of reconstitution buffer lacking glycerol. For the fluorescently labeled v-SNARE vesicles, 700 μl of the sample was overlaid with 1 ml of 30% (w/v) Nycodenz dissolved in reconstitution buffer followed by 1.5 ml of reconstitution buffer lacking glycerol. The samples were centrifuged at 200,000*g* at 4 °C for 4 h. The vesicles were harvested from the 0/30% Nycodenz interface.

2.3. Liposome flotation assay

The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with 180 pmol of MBP-tomosyn fragment in 80 μl of reconstitution buffer at 4 °C for 2 h. Then an equal volume of 80% (w/v) Nycodenz dissolved in reconstitution buffer was added and diluted with 40% (w/v) Nycodenz dissolved in reconstitution buffer to a final volume of 500 μl. The samples were overlaid with 800 μl of 35% (w/v) Nycodenz dissolved in reconstitution buffer, 800 μl of 30% (w/v) Nycodenz dissolved in reconstitution buffer, and 250 μl of reconstitution buffer lacking glycerol, and subjected to centrifugation at 200,000*g* at 4 °C for 4 h. The gradients were fractionated from the top (200 μl fractions) and the fractions were subjected to SDS–PAGE followed by CBB staining. The t-SNARE vesicles and the bound protein were recovered in Fractions 1–3.

2.4. Liposome fusion assay

The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with or without the various amounts of MBP-tomosyn, MBP-Tail-VLD, MBP-VLD, or MBP-ΔN-VLD in 56 μl of reconstitution buffer at 4 °C for 2 h. For the negative control, the control vesicles alone were incubated in 56 μl of reconstitution buffer at 4 °C for 2 h. The samples were mixed with 8 μl of the fluorescently labeled v-SNARE vesicles on ice, and immediately 60 μl of the samples were placed in a 96-well plate (Nunc) on an ice-cold metal block, followed by overlaying with 50 μl of mineral oil (Sigma). Increase of the NBD fluorescence upon lipid mixing was monitored at 1 min interval using the fluorimeter (Fluoroskan Ascent FL, Thermo Scientific) equilibrated to 37 °C with the filters set at 460 nm (excitation) and 538 nm (emission). Then, 10 μl of

2.5% (w/v) Triton X-100 was added to terminate the fusion reactions and dequench the NBD fluorescence. To normalize the fusion-dependent fluorescence, the lowest NBD fluorescent signals and the maximum signals after Triton X-100 addition were set to 0% and 100% fluorescence, respectively.

2.5. GST pull-down assay for the binding of the tail domain to the VLD

A 2000 pmol of GST-VLD, GST-ΔN-VLD, and GST were immobilized on 20 μl of glutathione Sepharose, respectively, and incubated with 1000 pmol of His-Tail in a buffer containing 20 mM

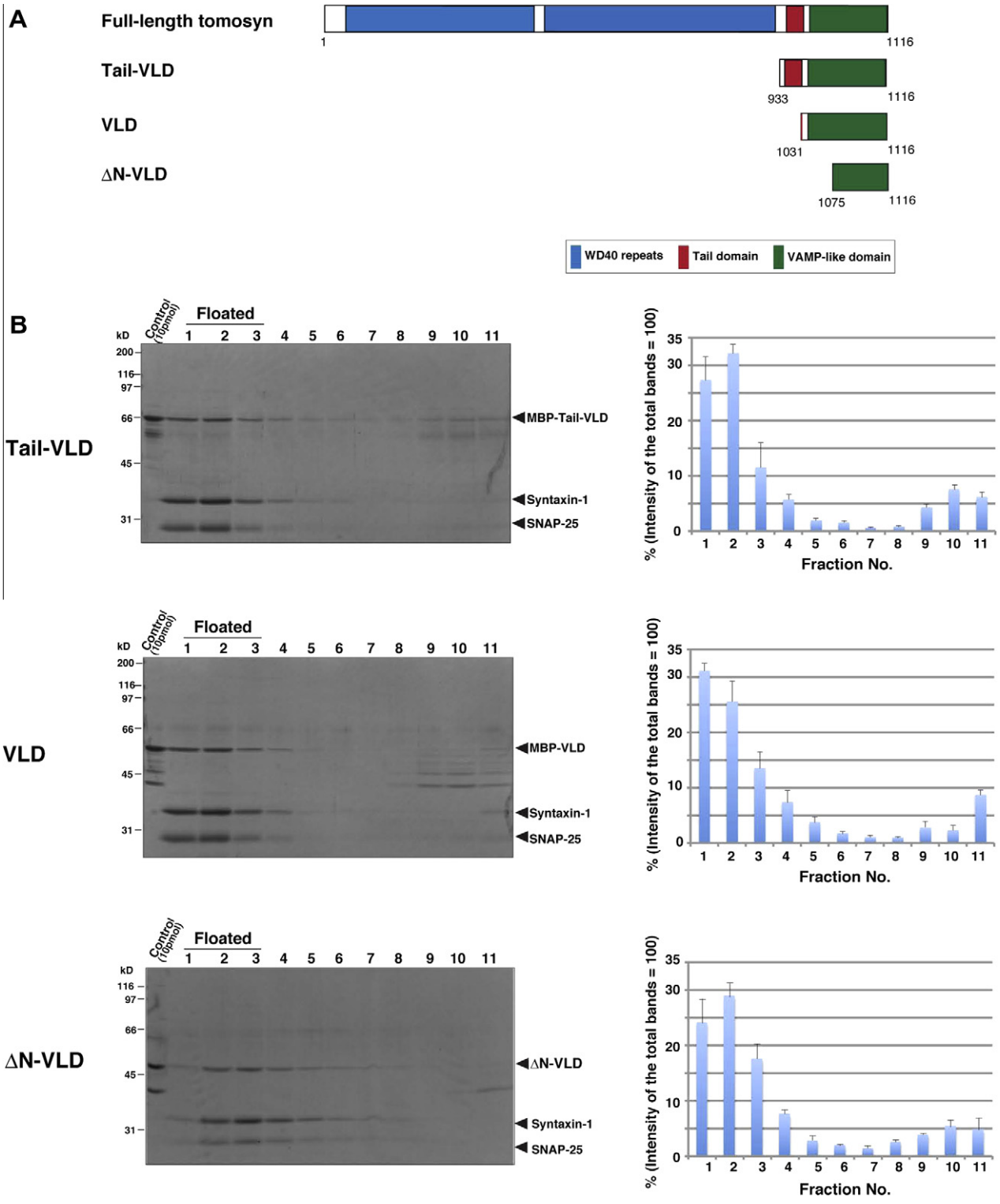

Fig. 1. No effect of the tail domain on binding of the VLD to t-SNAREs. (A) Schematic representation of tomosyn fragments used in this study. (B) Efficient binding of Tail-VLD, VLD, and ΔN-VLD to t-SNAREs on liposomes. The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with 180 pmol of MBP-VLD, MBP-Tail-VLD or MBP-ΔN-VLD to allow the tomosyn–SNARE complex formation on the vesicles, and then suspended in 40% Nycodenz. The samples were overlaid with 35% Nycodenz, 30% Nycodenz, and 0% Nycodenz, and subjected to ultracentrifugation. The gradients were fractionated from the top and subjected to SDS–PAGE followed by CBB staining. The t-SNARE vesicles and the bound proteins were floated and recovered in Fractions 1–3. The right panels show quantifications of the bindings of the tomosyn fragments to the t-SNARE vesicles. The results shown are representative of three independent experiments. Error bars represent SD.

Tris/HCl, pH 7.5, 20 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5% (w/v) Nonidet P-40 at 4 °C overnight. After being extensively washed with the buffer, the resins were boiled in an SDS sample buffer (60 mM Tris/HCl, pH 6.7, 3% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, and 5% (w/v) glycerol) for 5 min. The eluted proteins were subjected to SDS–PAGE followed by immunoblotting with an anti-His mAb (Novagen).

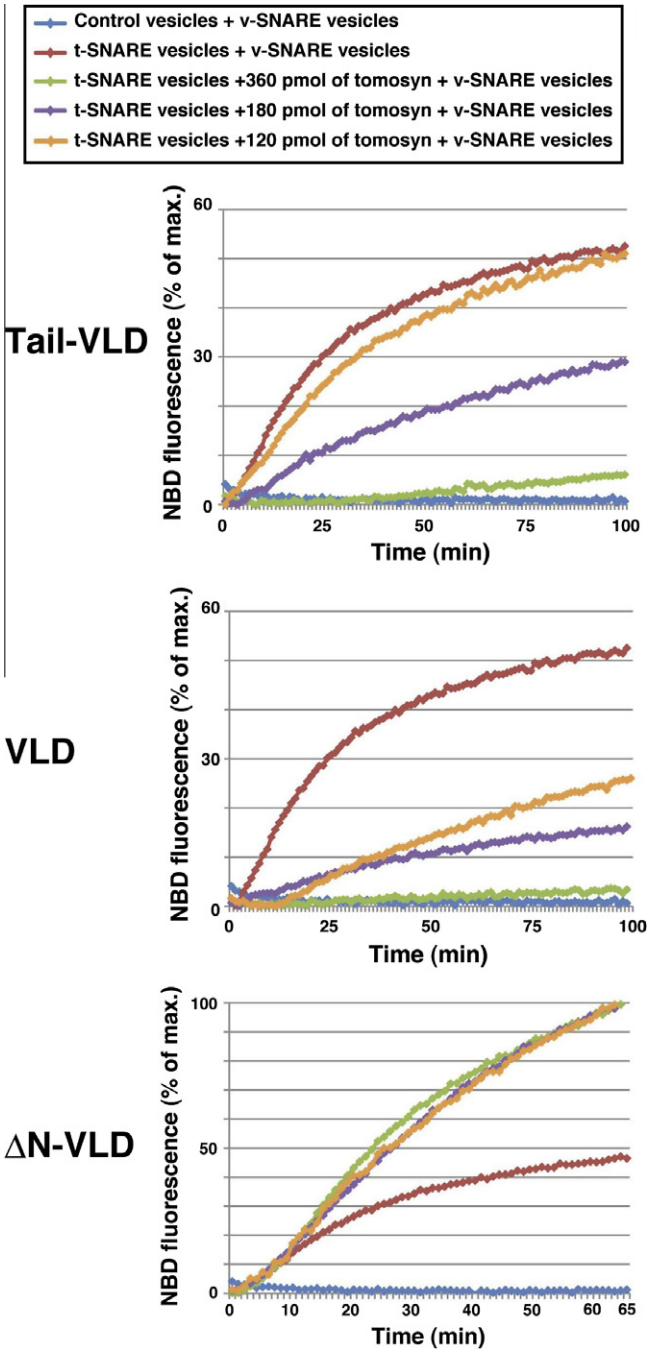

Fig. 2. Control of SNARE complex-mediated liposome fusion by the tail domain. The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with various amounts of MBP-VLD, MBP-Tail-VLD, or MBP-ΔN-VLD to allow the tomosyn–SNARE complex formation on the vesicles. Then, the samples were reacted with the fluorescently labeled v-SNARE vesicles. For the negative control reaction, the control vesicles, where no t-SNARE was embedded, were reacted with the fluorescently labeled v-SNARE vesicles. The liposome fusion was monitored by measuring the increase of NBD fluorescence. The results shown are representative of three independent experiments.

3. Results

3.1. The tail domain has no effect on the binding of tomosyn to t-SNAREs

Recent our electrophysiological study indicated that the inhibitory activity of tomosyn on neurotransmitter release is regulated by the tail domain binding to the VAMP-like domain (VLD) [16]. In this study, we employed the liposome fusion assay to better understand the mode of action of tomosyn. We made three kinds of MBP-fused tomosyn fragment, an MBP-fused fragment encompassing the VAMP-like domain (MBP-VLD), an MBP-fused fragment encompassing both the tail domain and the VLD (MBP-Tail-VLD), and an MBP-fused N-terminally truncated fragment of VLD (MBP-ΔN-VLD) (Fig. 1A). Firstly, we examined the bindings of the tomosyn fragments to t-SNAREs on liposomes by flotation analysis. The tomosyn fragments were incubated with liposomes bearing syntaxin-1 and SNAP-25 (referred to as t-SNARE vesicles hereafter), respectively, and the samples were subjected to Nycodenz gradient ultracentrifugation. Almost all of MBP-VLD was floated with the t-SNARE vesicles (Fig. 1B), indicating that MBP-VLD efficiently bound to t-SNAREs on the liposomes. Similarly, almost all of MBP-Tail-VLD and MBP-ΔN-VLD were floated with the t-SNARE vesicles. These results indicate that the tail domain unexpectedly has no effect on the VLD binding to t-SNAREs, and that the C-terminal half of the VLD is sufficient for the binding.

3.2. The tail domain regulates tomosyn displacement by VAMP2 for membrane fusion

We next examined the effects of VLD and Tail-VLD on the SNARE complex-mediated liposome fusion. The t-SNARE vesicles were incubated with various amounts of MBP-VLD and MBP-Tail-VLD, respectively, to allow the tomosyn–SNARE complex formation on the vesicles, and reacted with liposomes bearing VAMP2 (referred to as v-SNARE vesicle hereafter), where a quenched mixture of NBD-DPPE and rhodamine-DPPE was incorporated. Fusion between the t-SNARE vesicles and v-SNARE vesicles was measured by monitoring NBD fluorescence increase caused by dilution of the fluorescent lipids upon fusion as previously described [3].

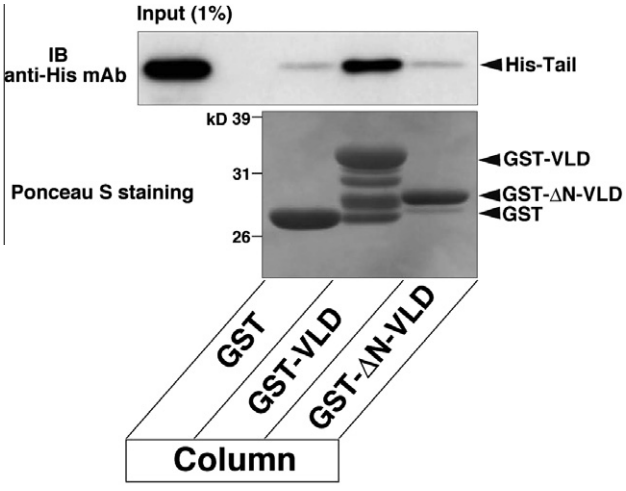

Fig. 3. Binding of the tail domain to the N-terminal half of the VLD. A 2000 pmol of GST-VLD, GST-ΔN-VLD, and GST were immobilized on glutathione Sepharose, respectively, and incubated with 1000 pmol of His-Tail. After being extensively washed, the bound proteins were eluted in the SDS sample buffer with boiling. A 1% of input and 13% of the eluted proteins were subjected to SDS–PAGE followed by immunoblotting with the anti-His mAb. The upper panel shows the immunoblotting with the anti-His mAb, and the lower panel shows the blotted membrane stained with Ponceau S.

MBP-VLD inhibited the SNARE complex-mediated liposome fusion in a dose-dependent manner (Fig. 2). MBP-Tail-VLD moderately inhibited the liposome fusion relative to MBP-VLD in the all tested concentrations, indicating that the tail domain decreases the inhibitory activity of the VLD on the SNARE complex-mediated membrane fusion. Given that the tail domain has no effect on the VLD binding to t-SNAREs (Fig. 1), these results indicate that the tail domain regulates tomosyn displacement by VAMP2 for membrane fusion.

3.3. Upon deletion of the tail domain-binding region, the VLD promotes membrane fusion

We similarly incubated the t-SNARE vesicles with various amounts of MBP-ΔN-VLD to allow the tomosyn–SNARE complex formation on the vesicles, and reacted with the v-SNARE vesicles. In contrast to the established role of t-SNARE sequestering of the VLD, MBP-ΔN-VLD significantly promoted the liposome fusion (Fig. 2). Given the efficient binding of ΔN-VLD to t-SNAREs (Fig. 1), this result indicates that ΔN-VLD enhances its displacement by VAMP2. We next examined whether the tail domain bound to ΔN-VLD. GST-fused ΔN-VLD (GST-ΔN-VLD), GST-fused VLD (GST-VLD) and GST alone were immobilized on glutathione Sepharose, respectively, and incubated with histidine-tagged fragment encompassing the tail domain (His-Tail). After being washed extensively, the bound proteins were eluted with the SDS sample buffer and subjected to SDS–PAGE followed by immunoblotting with the anti-His mAb. His-Tail bound to GST-VLD but not to GST-ΔN-VLD and GST alone (Fig. 3), indicating that the tail domain binds to the N-terminal half of the VLD. Together, these results indicate that the C-terminal half of the VLD serves as a placeholder for VAMP2 to promote membrane fusion and that is repressed by the N-terminal tail domain-binding region.

3.4. The inhibitory activity of full-length tomosyn is balanced by the reciprocal bindings of the tail domain

We recently reported that the tail domain reciprocally binds to the N-terminal WD40 repeats and the VLD [16]. To further confirm tomosyn displacement by VAMP2, we finally examined effect of full-length tomosyn on membrane fusion. An MBP-fused full-length tomosyn (MBP-tomosyn) was incubated with t-SNARE vesicles and the samples were subjected to Nycodenz gradient ultracentrifugation. Almost all of MBP-tomosyn was floated with the

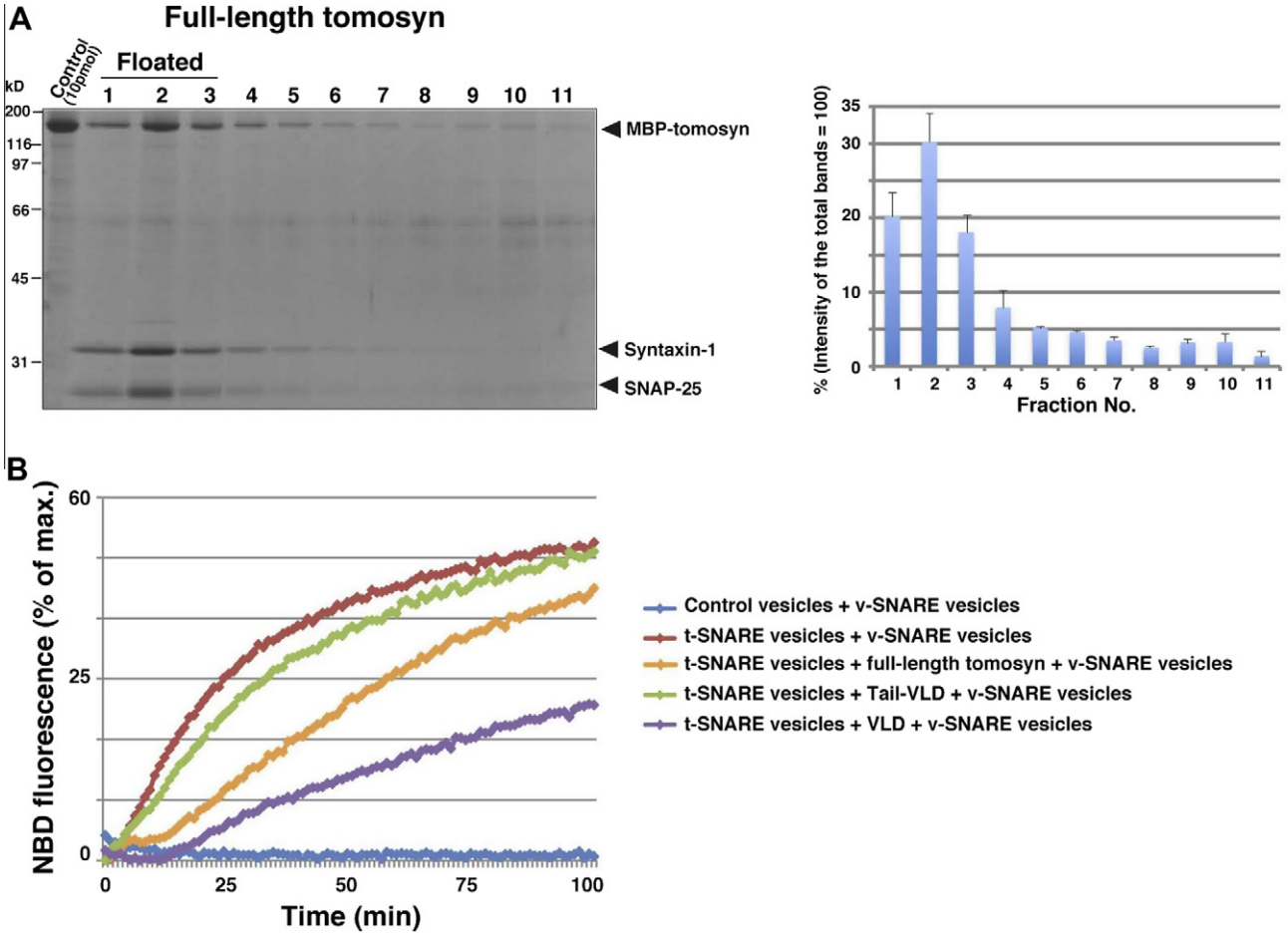

Fig. 4. The inhibitory activity of full-length tomosyn is balanced by the reciprocal bindings of the tail domain. (A) Efficient binding of full-length tomosyn to t-SNAREs on liposomes. The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with 180 pmol of MBP-tomosyn to allow the tomosyn–SNARE complex formation on the vesicles, and subjected to Nycodenz gradient flotation as done in Fig. 1B. The t-SNARE vesicles and the bound proteins were floated and recovered in Fractions 1–3. The right panel shows quantification of the binding of MBP-tomosyn to the t-SNARE vesicles. The result shown is representative of three independent experiments. Error bars represent SD. (B) Effect of full-length tomosyn on SNARE complex-mediated liposome fusion. The t-SNARE vesicles, where 360 pmol each of t-SNAREs was embedded, were incubated with 120 pmol of MBP-tomosyn, MBP-VLD, MBP-Tail-VLD, or MBP-ΔN-VLD to allow the tomosyn–SNARE complex formation on the vesicles. Then, the samples were reacted with the fluorescently labeled v-SNARE vesicles. The liposome fusion was monitored in the same manner as done in Fig. 2. The result shown is representative of three independent experiments. It is noted that MBP-Tail-VLD, MBP-VLD, and MBP-ΔN-VLD are repetition of Fig. 2, and the data are from the single experiment where MBP-tomosyn, MBP-Tail-VLD, MBP-VLD, and MBP-ΔN-VLD were reacted with the same lot of vesicles.

t-SNARE vesicles (Fig. 4A), indicating that full-length tomosyn bound to t-SNAREs on the liposomes as efficiently as Tail-VLD and VLD did. The t-SNARE vesicles were incubated with equal amount of MBP-tomosyn, MBP-Tail-VLD, or MBP-VLD, and reacted with the v-SNARE vesicles. MBP-tomosyn inhibited the liposome fusion more than MBP-Tail-VLD and less than MBP-VLD (Fig. 4B). We observed no binding of an MBP-fused fragment encompassing the N-terminal WD40 repeats (MBP-WD40 repeats) to t-SNARE vesicles and no effect of MBP-WD40 repeats on liposome fusion (Supplementary Fig. 1), indicating that the N-terminal WD40 repeats itself has no activity on SNARE complex-mediated membrane fusion. Therefore, the inhibitory activity of full-length tomosyn was accordingly balanced by the reciprocal intramolecular bindings of the tail domain. Collectively, these results indicate that the tail domain controls membrane fusion through tomosyn displacement by VAMP2.

4. Discussion

We showed here that the tail domain of tomosyn controlled membrane fusion though tomosyn displacement by VAMP2. Interestingly, deletion of the tail domain-binding region in the VLD retained the binding to t-SNAREs and significantly promoted liposome fusion. Contrary to the notion that the tomosyn–SNARE complex is a dead-end complex to inhibit neurotransmitter release, these results suggest a novel mechanism of tomosyn that controls synaptic vesicle fusion positively by the tail domain binding. While tomosyn potently inhibits membrane fusion by sequestering t-SNAREs through the VLD, the tail domain binding to the VLD may mask the N-terminal half of the VLD, allowing tomosyn to be a placeholder for VAMP2 to promote membrane fusion. In general, SNARE complex drives liposome fusion much slower than synaptic vesicle fusion [3], suggesting involvement of a special mechanism(s) to promote membrane fusion in the synaptic vesicle fusion. Recent studies have suggested that a regulated assembly of syntaxin-1 and SNAP-25 is a key to accelerate membrane fusion. Syntaxin-1 and SNAP-25 predominantly form a stable complex at 2:1 stoichiometry where the second syntaxin-1 occupies the position of VAMP2 [10,11]. However, VAMP2 prefers 1:1 syntaxin-1/SNAP-25 complex for the fast membrane fusion [11]. Indeed, the previous study demonstrated that a C-terminal half fragment of VAMP2 which serves as an artificial acceptor for 1:1 syntaxin-1/SNAP-25 complex accelerates liposome fusion [11]. Importantly, the artificial VAMP2 fragment is highly homologous to ΔN-VLD used in this study. Therefore, upon binding of the tail domain to the VLD, tomosyn may accelerate membrane fusion by serving as an endogenous acceptor for the 1:1 syntaxin-1/SNAP-25 complex. In contrast to the established inhibitory role of tomosyn, some results in the superior cervical ganglion neurons and the insulin-secreting cell line have suggested positive roles of tomosyn in vesicle fusion [22,23]. The above-mentioned mechanism that tomosyn is a placeholder for VAMP2 is likely to account for the positive effect of tomosyn. We recently reported that the binding affinity between the tail domain and the VLD is very weak [16]. In agreement, Tail-VLD is not sufficient for promoting liposome fusion by itself (Fig. 2). These results suggest a factor(s) are still missing to exert the positive effect of tomosyn. We previously reported that tomosyn is a substrate of protein kinase A (PKA) [22]. PKA phosphorylates the N-terminal WD40 repeats, decreasing tomosyn binding to syntaxin-1. Therefore, the PKA phosphorylation may enhance the tail domain binding to the VLD, leading to robust exertion of the positive effect. Tomosyn might be dominantly phosphorylated in the superior cervical ganglion neurons and the insulin-secreting cells. While we showed here that the tail domain did not affect the VLD binding to t-SNAREs on the liposomes, we previously demonstrated that the tail domain moderately decreases assembly of the VLD, the cytoplasmic domain of syntaxin-1 and SNAP-25 under membrane-free condition [16]. These results suggest that t-SNAREs embedded in membrane are preferable targets for tomosyn, although it remains unclear how the membranous environment affects the tomosyn–SNARE complex formation. Further studies will be required to address these concerns.

In summary, the tail domain of tomosyn controls membrane fusion through tomosyn displacement by VAMP2.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.07.026.

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