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Membrane Lipids Influence Protein Complex Assembly−Disassembly

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The chemistry of life processes is governed at the molecular level. For example, membrane-directed self-assembly of a supramolecular ring complex is required for the establishment of continuity between opposing membrane compartments in cells.[1,2] Neurotransmission and the secretion of hormones or digestive enzymes all involve fusion of cellular membranes. At the nerve terminal, fusion involves the conserved target membrane proteins SNAP-25 and syntaxin 1A, termed t-SNAREs, and the synaptic vesicle-associated membrane protein VAMP2, termed v-SNARE.[3−5] In the presence of Ca^{2+}, when a v-SNARE-reconstituted liposome meets a t-SNARE-reconstituted vesicle, the SNAREs in opposing membranes interact and self-assemble into a ring, establishing continuity between the compartments.[1,2] In the presence of ATP, this highly stable membrane-directed and self-assembled SNARE complex can undergo disassembly in the presence of the soluble ATPase *N*-ethylmaleimide-sensitive factor (NSF).[8,9] Cholesterol and lysophosphatidylcholine (LPC) are known to contribute to the negative and positive curvature of the cell membrane.[6,7] Since cholesterol and LPC have been implicated in the promotion and inhibition of membrane fusion, respectively,[10] their influence on membrane-directed assembly and disassembly of the t-/v-SNARE ring complex was hypothesized. To test this hypothesis, the structure of the t-/v-SNARE complex at nanometer resolution was determined using atomic force microscopy (AFM), and at the molecular level, the secondary structures of SNAREs and their complex in membranes containing either cholesterol or LPC were determined using circular dichroism (CD) spectroscopy.[9]

Since vesicle size influences membrane curvature, a uniform vesicle population prepared using a published[2] extrusion method was used for the entire study. Two sets of 50 nm diameter liposomes, one set containing cholesterol and the other LPC, were reconstituted with either t-SNAREs or v-SNARE for use (Figure 1). Surprisingly, examination at the nanometer level using AFM demonstrated significant ($p < 0.001$) differences in SNARE ring size formed in the presence of cholesterol as opposed to LPC. SNARE ring complexes formed using cholesterol-associated vesicles were found to be 6.89 nm in size, which is ∼11% smaller than the 7.746 nm size obtained using LPC-containing vesicles. CD spectroscopy showed that SNARE ring complexes formed in the presence of cholesterol (Figure 2A) and LPC (Figure 2B) further demonstrate profound differences (Table 1). As previously determined,[9] our results (Table 1) reveal high α-helical content in t-SNARE and t-/v-SNARE complexes. However, in the presence of NSF and ATP, peaks at 208 and 222 nm characteristic of α-helical secondary structure are abolished in the cholesterol groups (Figure 2 and Table 1). CD spectra of membrane-associated v-SNARE display little signal, as previously demonstrated;[9] however, v-SNARE reconstituted in liposomes containing cholesterol display CD signals for α-helical content. In contrast, the LPC groups exhibit no signal for α-helical content.

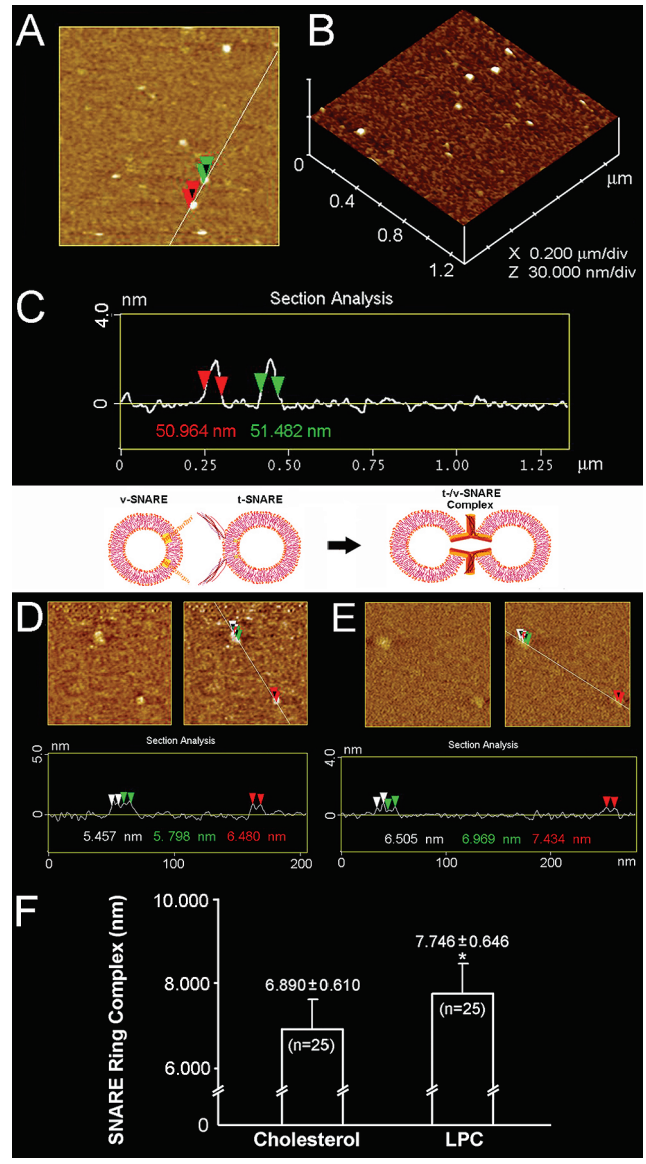

Figure 1. Representative AFM micrographs of ∼50 nm diameter liposomes and the t-/v-SNARE ring complexes formed when such cholesterol- or LPC-containing t-SNARE and v-SNARE proteoliposomes meet. (A−C) 50−53 nm diameter cholesterol-containing liposomes. Similarly sized LPC-containing vesicles were prepared and observed using AFM (data not shown). (D, F) 6.89 ± 0.61 nm t-/v-SNARE ring complexes formed when ∼50 nm diameter t-SNARE−cholesterol liposomes interact with 50 nm v-SNARE−cholesterol vesicles. (E, F) Similar 7.746 ± 0.646 nm t-/v-SNARE ring complexes formed by replacing cholesterol with LPC. * indicates $p < 0.001$.

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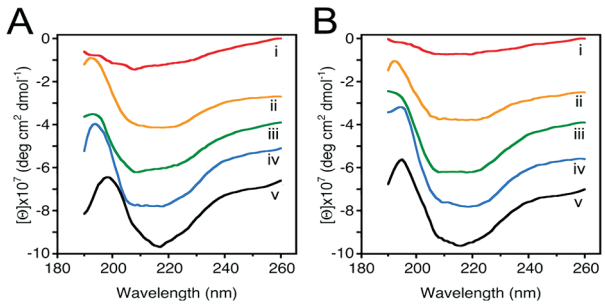

Figure 2. CD data reflecting structural changes to SNAREs associated with liposomes containing (A) cholesterol and (B) LPC. Structural changes following the assembly and NSF−ATP-induced disassembly of the t-/v-SNARE complex are further shown: (i) v-SNARE; (ii) t-SNAREs; (iii) t-/v-SNARE complex; (iv) t-/v-SNARE + NSF; and (v) t-/v-SNARE + NSF + 2.5 mM ATP. CD spectra were recorded at 25 °C in 5 mM sodium phosphate buffer (pH 7.5) at a protein concentration of 25 μM. In each experiment, scans were averaged per sample for enhanced signal-to-noise, and data were acquired on duplicate independent samples to ensure reproducibility. The decrease in α-helicity in the cholesterol groups as opposed to the LPC groups following exposure of the t-/v-SNARE complex to NSF-ATP should be noted (Table 1).

Table 1. Secondary Structural Fit Parameters of SNARE Complex Formation and Dissociation[a]

	cholesterol group					LPC group				
	liposome + cholesterol (100*f*)					liposome + LPC (100*f*)				
protein[b]	α	*β*	O	U	fit[c]	α	*β*	O	U	fit[c]
v-SNARE	21	28	0	51	0.11	0	23	32	44	0.38
t-SNARE	20	18	11	51	0.17	21	22	0	57	0.22
t-/v-SNARE	27	29	0	45	0.10	26	20	0	55	0.22
t-/v-SNARE + NSF	18	2	6	75	0.26	22	17	3	58	0.25
t-/v-SNARE + NSF + ATP	0	21	40	39	0.20	20	3	0	76	0.10

[a] Abbreviations used: *f*, fraction of residues is a given conformational class; α, α-helix; *β*, *β*-sheet; O, other (sum of turns, distorted helix, distorted sheet); U, unordered. [b] Protein constructs: v-SNARE (VAMP2); t-SNAREs (SNAP-25 + syntaxin 1A); NSF, *N*-ethylmaleimide-sensitive factor; ATP, adenosine triphosphate. [c] Fit: goodness-of-fit parameter expressed as normalized spectral fit standard deviation (nm).

This cholesterol effect on v-SNARE could be due to a weak signal detected by the spectrometer or to the limitations of the GlobalWorks software (Olis) in fitting such data. The primary purpose of analyzing and presenting the v-SNARE data was to ensure that the protein did not provide abnormal signals that could influence the CD spectroscopy of the t-/v-SNARE complex.

In both the cholesterol and LPC groups, the overall spectroscopic profiles of t-SNAREs and t-/v-SNARE complexes are similar to the previously reported studies[9] using t- and t-/v-SNARE control liposomes (without cholesterol or LPC molecules).[9] However, the NSF−ATP-induced disassembly of the SNARE complex is similar in the t-/v-SNARE control liposome group[9] and the cholesterol liposome group (Figure 2) in comparison with the LPC liposome group. Addition of ATP to the t-/v-SNARE complex in the presence of NSF demonstrated a decrease in α-helicity in the cholesterol groups (Figure 2). Interestingly however, in the presence of LPC, ATP-induced SNARE complex disassembly was abrogated. Similarly, in agreement with earlier studies,[9] addition of ATP to the t-/v-SNARE complex in the presence of NSF showed little or no change in the *β*-sheet structures in the cholesterol groups (Figure 2), but in the LPC groups, NSF−ATP induced nearly complete disassembly of the *β*-sheet structures within the SNARE complex. Since the presence of LPC blocks NSF−ATP-induced disassembly of α-helical contents in the t-/v-SNARE complex, these results are in agreement with previous findings, which support LPC to be a membrane fusion inhibitor.[11] Additionally, in the presence of LPC, the inability of the SNARE complex to disassemble, and therefore be able to participate in a new round of docking and fusion, would severely limit membrane fusion during cell secretion. The results of this study provide evidence for the critical role of specific lipids in the structure and function of membrane proteins.

In summary, we have reported for the first time that membrane-curvature-influencing lipids profoundly influence SNARE complex size and its disassembly. This influence is evident in the 11% smaller t-/v-SNARE ring complexes formed using 50 nm cholesterol-associated vesicles as opposed to LPC-associated ones. As previously reported,[9] in membrane containing no cholesterol or LPC, NSF−ATP induces disassembly of the α-helical contents but not the *β*-sheet structures in the t-/v-SNARE complex. In contrast, in the presence of LPC, NSF−ATP induces disassembly of the *β*-sheet structures but not the α-helical contents in the SNARE complex. Previous studies indicate that the role of cholesterol in membrane fusion is indirect, centered on SNARE formation through cholesterol binding to synaptophysin, a calcium- and cholesterol-dependent vesicle-associated protein that forms a complex with synaptobrevin (VAMP), subsequently facilitating v-SNARE interaction with t-SNAREs.[12] In the present study, however, no synaptophysin was present to influence such interactions of cholesterol with SNAREs, and therefore, little or no effect of cholesterol is demonstrated on the α-helical and *β*-sheet content of membrane-associated SNAREs and the SNARE complex. Our findings further support the existence of a direct lipid−protein relationship to differentially modulate SNARE function within various cellular compartments. Modulating the concentration and distribution of such nonbilayer lipids at various membranes could regulate the degree and rate of membrane fusion and membrane-directed SNARE complex assembly−disassembly. Cells with higher membrane cholesterol levels would promote membrane fusion, while cells with increased membrane LPC content would facilitate secretory event longevity by inhibiting SNARE complex disassembly.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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