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Energy-Dependent Disassembly of Self-Assembled SNARE Complex: Observation at Nanometer Resolution Using Atomic Force Microscopy

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Numerous life processes involve the engagement and disengagement of biomolecules. Knowledge of how molecules interact to assemble and disassemble provides an understanding of biochemical reactions. For example, membrane fusion essential to several cellular processes, such as intracellular transport, hormone secretion, enzyme release, or neurotransmission, is mediated via the assembly and disassembly of a specialized set of proteins present in opposing bilayers.[1,2] Target membrane proteins SNAP-25 and syntaxin-termed t-SNAREs, and secretory vesicle-associated protein, or v-SNARE, are part of the conserved protein complex involved in fusion of opposing membranes.[1,2] It has been demonstrated that, in the presence of Ca^{2+}, t-SNAREs and v-SNARE in opposing bilayers interact and self-assemble[3] to form supramolecular ring complexes or channels, which help establish continuity between opposing bilayers.[4,5] The present study was undertaken to understand the disassembly of such self-assembled t-/v-SNARE complexes.

The soluble *N-ethylmaleimide-sensitive factor* (NSF), an ATPase, has been implicated in the disassembly of the t-/v-SNARE complex in the presence of ATP.[1,6] However, conformation by the direct physical observation and kinetics of this NSF−ATP-induced SNARE disassembly had not been demonstrated. In this study, using purified recombinant NSF, and t- and v-SNARE-reconstituted liposomes, the disassembly of the t-/v-SNARE complex was examined. Lipid vesicles ranging in size from 0.2 to 2 μm were reconstituted with either t-SNAREs or v-SNARE. Kinetics of association and dissociation of t-SNARE- and v-SNARE-reconstituted liposomes in solution, in the presence or absence of NSF, ATP, and AMP−PNP (the nonhydrolyzable ATP analogue), were monitored by right angle light scattering[5] (Figure 1A,B). Addition of NSF and ATP to the t-/v-SNARE vesicle mixture led to a rapid and significant increase in intensity of light scattering (Figure 1A,B), suggesting rapid disassembly of the SNARE complex and dissociation of vesicles. Dissociation of t-/v-SNARE vesicles occurs on a logarithmic scale that can be expressed by first-order equation, with rate constant $k = 1.1\ s^{-1}$ (Figure 1B). To determine whether NSF-induced dissociation of t- and v-SNARE vesicles is energy driven, experiments were performed in the presence and absence of ATP and AMP−PNP. No significant change with NSF alone or in the presence of NSF−AMP−PNP was observed (Figure 1C). These results demonstrate that t-/v-SNARE disassembly is an enzymatic and energy-driven process.

To further confirm the ability of NSF−ATP in the disassembly of the t-/v-SNARE complex, immunochemical studies were performed. It has been demonstrated that v-SNARE and t-SNAREs form an SDS-resistant complex.[6] NSF binds to SNAREs and forms a stable complex when locked in the ATP-bound state (ATP−NSF). Thus, in the presence of ATP+EDTA, VAMP antibody has been

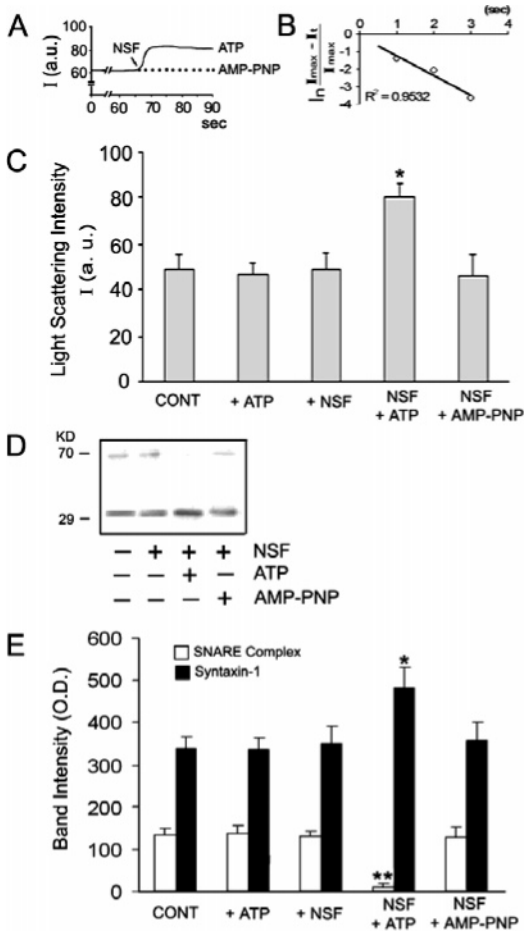

Figure 1. NSF−ATP-induced dissociation of t-SNARE- and v-SNARE-associated liposomes. (A) Real-time light scattering profiles of interacting t-SNARE and v-SNARE vesicles in solution in the presence and absence of NSF (depicted by arrow). In the presence of ATP, NSF rapidly disassembles the SNARE complex and dissociates SNARE vesicles represented as a rapid increase in light scattering. No change in light scattering is observed when ATP is replaced with the nonhydrolyzable analogue. AMP−PNP. (B) Kinetics of NSF-induced dissociation. The graph depicts first-order kinetics of vesicle dissociation elicited by NSF−ATP. (C) NSF requires ATP to dissociate vesicles. NSF in the presence of ATP dissociates vesicles (*$p < 0.05$, $n = 4$). However, NSF alone or NSF in the presence of AMP−PNP had no effect on the light scattering properties of the SNARE-associated vesicle ($p > 0.05$, $n = 4$). (D) When t- and v-SNARE vesicles are mixed in the presence or absence of ATP, NSF, NSF+ATP, or NSF+AMP−PNP, and resolved by SDS−PAGE followed by immunoblots using syntaxin-1 specific antibody, t-/v-SNARE disassembly was found to be complete only in the presence of NSF−ATP. (E) Densitometric scan of the bands reveals complete disassembly of the SNARE complex in the presence of NSF−ATP (**$p < 0.01$, $n = 4$).

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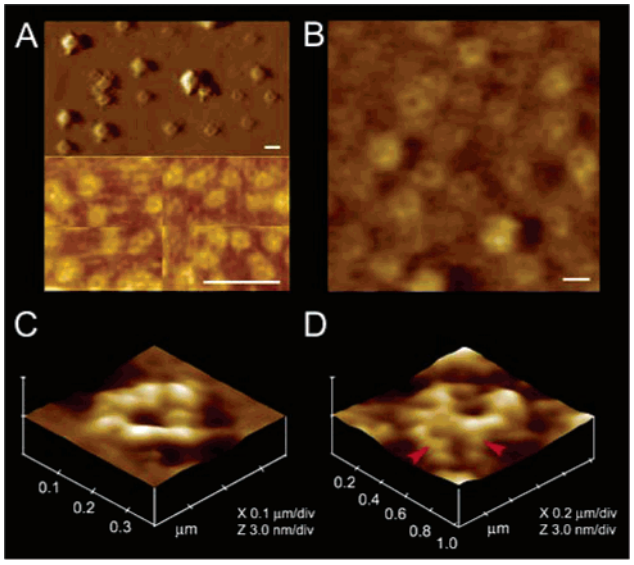

Figure 2. AFM micrographs of NSF−ATP-induced disassembly of the t-/v-SNARE ring complex. Representative AFM micrograph of t-/v-SNARE ring complexes, either large (top panel A) or small (bottom panel A), formed when large or small v-SNARE-reconstituted vesicles interact with a t-SNARE-reconstituted lipid membranes. Scale bar = 250 nm. (B) Disassembly of a large t-/v-SNARE complex. Scale bar = 250 nm. (C) High resolution of a t-/v-SNARE ring complex, and (D) a disassembled one. The red arrowheads indicate uncoiled t-/v-SNARE.

demonstrated to be able to immunoprecipitate this stable NSF−SNARE complex.[6] Therefore, in the present study, when t- and v-SNARE vesicles were mixed in the presence or absence of ATP, NSF, NSF+ATP, or NSF+AMP−PNP and resolved by SDS−PAGE followed by immunoblots using syntaxin-1 specific antibody, t-/v-SNARE disassembly was found to be complete only in the presence of NSF−ATP (Figure 1D,E).

To further confirm our findings (Figure 1), direct observation of the t-/v-SNARE complex disassembly was required. When purified recombinant t-SNAREs and v-SNARE in opposing bilayers interact and self-assemble[3] to form supramolecular ring complexes,[4] they disassembled when exposed to recombinant NSF and ATP, as observed at nanometer resolution using atomic force microscopy (AFM) (Figure 2). Earlier studies[4] demonstrate that, in contrast to SNARE-reconstituted liposomes (as would occur in the physiological state), soluble v- and t-SNAREs in the absence of membrane fail to assemble in any specific pattern or form such conducting pores. This would suggest that NSF may require the t-/v-SNARE complex to be arranged in a specific configuration or pattern for it to be able to bind and disassemble the complex in the presence of ATP. To test this hypothesis, t-SNAREs followed by v-SNARE, NSF, and ATP were added to a lipid membrane and continuously imaged in buffer by AFM (Figure 3). Our results demonstrate that SNARE complexes disassemble, even though such complexes fail to form the characteristic rings. Hence, NSF−ATP is able to disassemble SNARE complexes formed both when v-SNARE alone or v-SNARE-reconstituted liposomes are added to t-SNARE-reconstituted lipid membranes.

In summary, we have demonstrated for the first time that NSF−ATP is the minimal requirement for disassembly of the t-/v-SNARE complex. Hence, assembly and disassembly of SNARE complexes can be finely controlled. This has important implications in the regulated fusion and dissociation of artificial lipid membranes, which may find use in the controlled delivery of lipid-encapsulated drugs and in the transport of biomolecules.

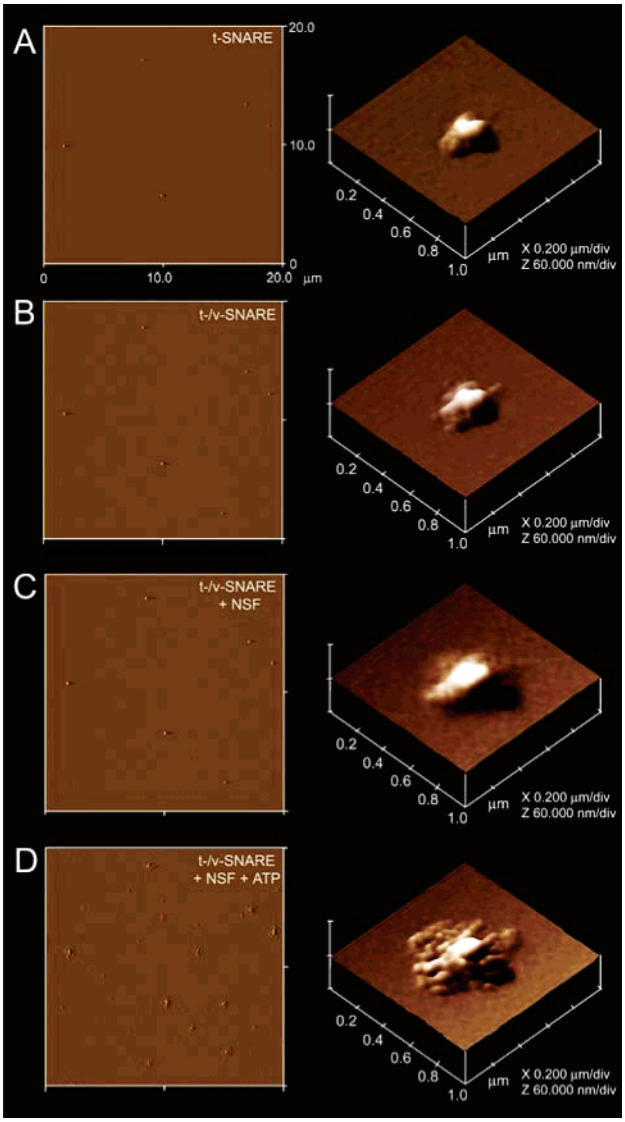

Figure 3. AFM micrographs of NSF−ATP-induced disassembly of the t-/v-SNARE complex formed when v-SNARE is added to a t-SNARE-reconstituted lipid membrane. The left panel A−D shows, at low resolution, the sequential AFM micrographs of 1 of 10 representative experiments, where v-SNARE is added to a t-SNARE-reconstituted lipid membrane, followed by NSF and then ATP. Note the dramatic disassembly of the SNARE complexes in D. The right panel shows, at higher resolution, the disassembly of one of such SNARE complexes.

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

References

(1) Söllner, T.; Whiteheart, S. W.; Brunner, M.; Erdjument-Bromage, H.; Geromanos, S.; Tempst, P.; Rothman, J. E. *Nature* **1993**, *362*, 318−324.
(2) Weber, T.; Zemelman, B. V.; McNew, J. A.; Westerman, B.; Gmachi, M.; Parlati, F.; Söllner, T. H.; Rothman, J. E. *Cell* **1998**, *92*, 759−772.
(3) Cho, W. J.; Jeremic, A.; Jena, B. P. *J. Am. Chem. Soc.* **2005**, *127*, 10156−10157.
(4) Cho, S. J.; Kelly, M.; Rognlien, K. T.; Cho, J.; Hörber, J. K.; Jena, B. P. *Biophys. J.* **2002**, *83*, 2522−2527.
(5) Jeremic, A.; Kelly, M.; Cho, J. A.; Cho, S. J.; Hörber J. K.; Jena, B. P. *Cell Biol. Int.* **2004**, *28*, 19−31.
(6) Jeong, E.-H.; Webster, P.; Khuong, C. Q.; Sattar, A. K. M. A.; Satchi, M.; Jena, B. P. *Cell Biol. Int.* **1998**, *22*, 657−670.

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