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Lipid-Induced Conformational Switch Controls Fusion Activity of Longin Domain SNARE Ykt6

Wenyu Wen,[1,3] Jiang Yu,[3] Lifeng Pan,[3] Zhiyi Wei,[3] Jingwei Weng,[1,2] Wenning Wang,[1,2] Yan Shan Ong,[4] Ton Hoai Thi Tran,[4] Wanjin Hong,[4] and Mingjie Zhang[1,3,*]
[1]Institutes of Biomedical Sciences
[2]Department of Chemistry
Fudan University, Shanghai, P.R. China
[3]Department of Biochemistry, Molecular Neuroscience Center, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, P.R. China
[4]Cancer and Developmental Cell Biology Division, Institute of Molecular and Cell Biology, Singapore 138673, Singapore
*Correspondence: mzhang@ust.hk
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SUMMARY

While most SNAREs are permanently anchored to membranes by their transmembrane domains, the dually lipidated SNARE Ykt6 is found both on intracellular membranes and in the cytosol. The cytosolic Ykt6 is inactive due to the autoinhibition of the SNARE core by its longin domain, although the molecular basis of this inhibition is unknown. Here, we demonstrate that unlipidated Ykt6 adopts multiple conformations, with a small population in the closed state. The structure of Ykt6 in complex with a fatty acid suggests that, upon farnesylation, the Ykt6 SNARE core forms four α helices that wrap around the longin domain, forming a dominantly closed conformation. The fatty acid, buried in a hydrophobic groove formed between the longin domain and its SNARE core, is essential for maintaining the autoinhibited conformation of Ykt6. Our study reveals that the posttranslationally attached farnesyl group can actively regulate Ykt6 fusion activity in addition to its anticipated membrane-anchoring role.

INTRODUCTION

In eukaryotic cells, the dynamic trafficking of proteins and lipids between organelles is closely linked to membrane-bound vesicles that pinch off from one membrane and fuse with another (Jahn and Scheller, 2006; Südhof and Rothman, 2009). The specific targeting and fusion of different classes of transport vesicles to their distinct membrane destinations, which is essential to ensure the integrity and functions of organelles, relies on the precise pairing of cognate soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) anchored separately to the two membranes involved (Nicholson et al., 1998; Söllner et al., 1993; Weber et al., 1998). SNARE activity is governed in part by the conformational state of SNARE proteins. Most SNARE proteins contain three domains: a variable N-terminal regulatory domain, a conserved central 60–70 amino acid "SNARE core" that mediates the self-assembly of the four-helix-bundle SNARE core complex, and a C-terminal transmembrane domain (Sutton et al., 1998). In syntaxin SNAREs syntaxin-1 and Sso1p, the N-terminal three-helix-bundle Habc domain folds back and packs extensively with the SNARE core helix, preventing the SNARE core from freely forming the fusion-competent SNARE core complex (Dulubova et al., 1999; Misura et al., 2000; Munson et al., 2000; Nicholson et al., 1998). The N-terminal regulatory domain-mediated sequestration of the SNARE core is also observed in the nonsyntaxin SNARE Ykt6 (Hasegawa et al., 2004; Rossi et al., 2004; Tochio et al., 2001), although the molecular details of this autoinhibition are unclear.

Ykt6 is the most conserved and versatile SNARE (Rossi et al., 2004). Yeast Ykt6 is an essential protein involved in multiple membrane fusion reactions at the Golgi, vacuoles, and endosomes (Dilcher et al., 2001; Kweon et al., 2003; Lupashin et al., 1997; McNew et al., 1997; Meiringer et al., 2008; Ungermann et al., 1999). Mammalian Ykt6 is highly enriched in animal brains, and it forms specialized punctuates of unknown compartments in neurons (Hasegawa et al., 2003, 2004). In other mammalian cells, Ykt6 is found at the Golgi, perinuclear space, and cytosols (Fukasawa et al., 2004; Zhang and Hong, 2001). Unlike most other SNARE proteins, Ykt6 does not contain a transmembrane domain for stable membrane association. Instead, it contains a C-terminal "CCAIM" motif that can be palmitoylated at the first cysteine and farnesylated at the second cysteine (Figure S1A) (Fukasawa et al., 2004; McNew et al., 1997). A striking feature of Ykt6 is that it exists in both membrane-bound and soluble cytosolic pools, and functional Ykt6 requires the cycling of the protein between membranes and cytosol (Fukasawa et al., 2004; Hasegawa et al., 2003; McNew et al., 1997; Meiringer et al., 2008; Zhang and Hong, 2001). The stable membrane association requires both lipidations, and only membrane-anchored Ykt6 is fusion active (Fukasawa et al., 2004; Meiringer et al., 2008). The farnesylation of Ykt6 occurs posttranslationally and is essentially irreversible (Resh, 2006). Palmitoylation, instead, is a reversible and dynamic process (Dietrich and Ungermann, 2004; Linder and Deschenes, 2007; Meiringer et al., 2008). Thus, one might envision that single-lipidated (i.e., farnesylation at Cys195) Ykt6 exists in the cytosol and that cytosolic Ykt6 adopts an autoinhibited conformation via farnesyl-dependent interaction between its SNARE core and longin domain. The