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Brief Communication

Two Molecules of Lobophorolide Cooperate to Stabilize an Actin Dimer Using Both Their "Ring" and "Tail" Region

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SUMMARY

Actin filament-disrupting marine macrolides are promising templates from which to design therapeutics against cancer and other diseases that co-opt the actin cytoskeleton. Typically, these macrolides form either a 1:1 or 2:1 actin-macrolide complex where their aliphatic side chain, or "tail," has been reported to convey the major determinant of cytotoxicity. We now report the structure of the marine macrolide lobophorolide bound to actin with a unique 2:2 stoichiometry in which two lobophorolide molecules cooperate to form a dimerization interface that is composed entirely of the macrolide "ring" region, and each molecule of lobophorolide interacts with both actin subunits via their ring and tail regions to tether the subunits together. This binding mode imposes multiple barriers against microfilament stability and holds important implications for development of actin-targeting drugs and the evolution of macrolide biosynthetic enzymes.

INTRODUCTION

Proper regulation of actin polymerization is central to many processes in eukaryotic cells (Pollard and Borisy, 2003) and a large number of diverse natural products have been found that bind to actin and disrupt its polymerization dynamics, leading to high cytotoxicity in numerous cell types (Allingham et al., 2006). Many actin-binding compounds are monomeric macrolides that consist of a highly variable 24- to 26-membered macrolactone ring with a long aliphatic side chain (tail) terminating with an *N*-methyl-vinylformamide moiety (see Figure S1 available online). These compounds bind the barbed end of actin to form a 1:1 actin-macrolide complex that disrupts longitudinal interactions between adjacent actin filament subunits, allowing sequestration of globular actin (G-actin), severing of filamentous actin (F-actin), and capping of filament ends (Allingham et al., 2005; Klenchin et al., 2003). X-ray crystal structures of these macrolides bound to actin have revealed common actin-binding interfaces for defined regions of different macrolides and have provided some molecular explanations for their effects (Allingham et al., 2005; Hirata et al., 2006; Klenchin et al., 2003). Most of the observed actin-binding interface commonality among different macrolides involves the tail region, suggesting that the tail is a major contributor to their high affinity toward actin and cytotoxic functionality (Allingham et al., 2005; Hirata et al., 2006). With this information, synthetic mimetics comprising mainly the tail component of these macrolides have recently been developed with the potential to act as therapeutics for diseases that co-opt the actin cytoskeleton, such as cancer metastasis and certain microbial infections (Perrins et al., 2008). A deeper understanding of the contributions of the ring component to their inhibition of actin polymerization could guide refinement and elaboration of these mimetics and will rely on structure-function analysis of structurally unprecedented compounds that display potent cytotoxicity.

The marine sponge *Theonella swinhoei* produces a barbed end binding macrolide, named swinholide, that consists of a 44-membered dimeric cyclic lactone possessing two identical pyrone ring-terminated side chains, giving the molecule a 2-fold axis of symmetry (Figure 1A) (Kobayashi et al., 1990). As a result, swinholide forms an actin-macrolide complex with 2:1 stoichiometry in which each side chain accesses the barbed end cleft of a different actin molecule (Bubb et al., 1995; Klenchin et al., 2005). Interestingly, the brown alga *Lobophora variegata* produces a macrolide called lobophorolide that is essentially half of the dimeric swinholide (Figure 1A) (Kubanek et al., 2003). It consists of a 22-membered macrolactone ring attached to a pyrone ring-terminated aliphatic side chain, and thus is structurally unprecedented relative to the other monomeric macrolides described above. Both lobophorolide and swinholide display sub-μM antifungal activity and are highly cytotoxic to a variety of cancer cell lines, where swinholide's cytotoxic activities are dependent on the integrity of its ring structure (Kobayashi et al., 1994; Kubanek et al., 2003). Given its similarity to a portion of swinholide, lobophorolide has been postulated to be a barbed end targeting macrolide (Allingham et al., 2006); however, this has not been confirmed. To elucidate the basis for lobophorolide's cytotoxicity, we determined its structure bound to G-actin at 2.0 Å resolution by X-ray crystallography and analyzed its effects on purified actin polymers in vitro.

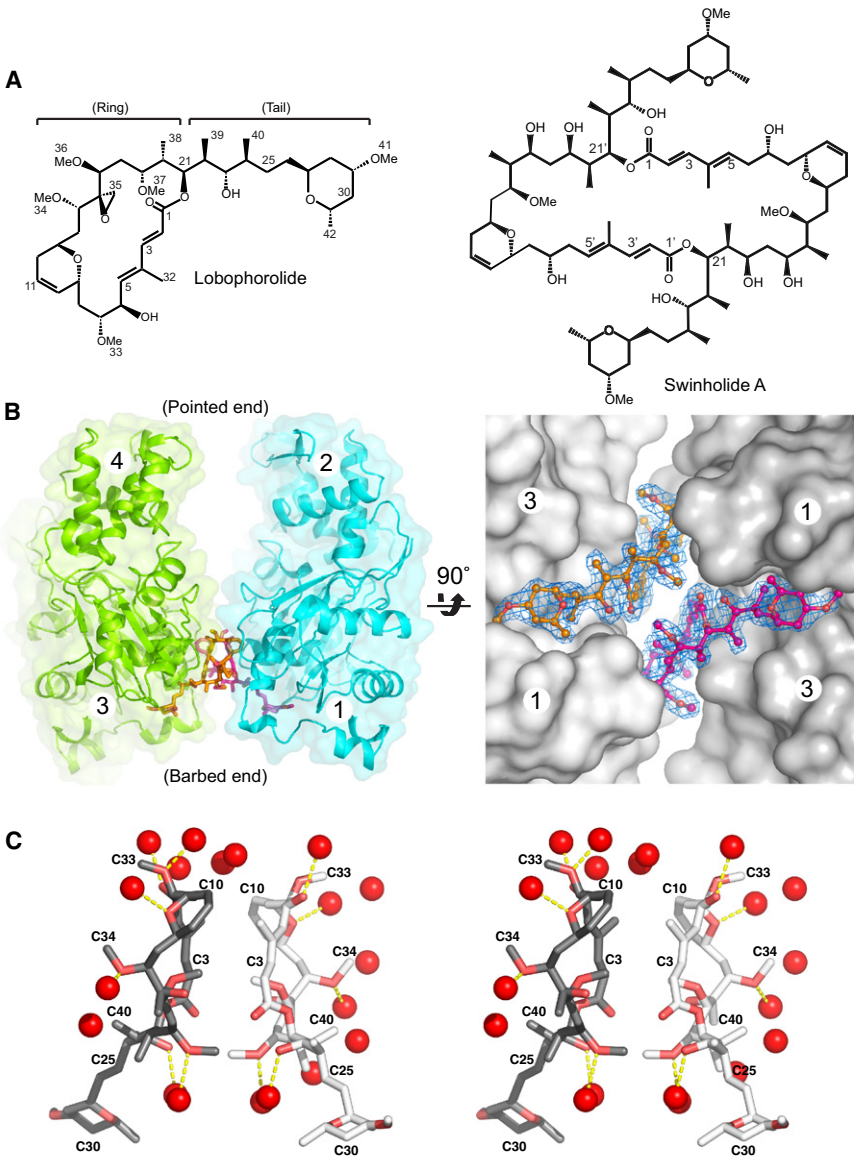

Figure 1. Chemical and Actin-Bound Structures of Lobophorolide

(A) The "ring" and "tail" components of lobophorolide are indicated. The chemical structure of swinholide A is shown for comparison.

(B) Two actin subunits (green and cyan) are stabilized as a complex with 2-fold rotational symmetry by two lobophorolide molecules (magenta and orange sticks). Subdomains 1 to 4 are labeled. The F_o-F_c electron density omit map contoured at 3 σ for each lobophorolide molecule is shown.

(C) Stereo view of the two lobophorolide molecules and nearby waters (red spheres) shows the exclusion of water molecules at the interface formed by their macrolactone rings. Dotted lines indicate bonds with waters.

RESULTS AND DISCUSSION

Overview of the Actin-Lobophorolide Complex

The asymmetric unit of the actin-lobophorolide crystal contains a complex in which two lobophorolide molecules mediate formation of a nonphysiological actin dimer with noncrystallographic 2-fold rotational symmetry (Figure 1B; Table S1). Electron density maps for both lobophorolide molecules are unambiguous and are in agreement with the stereochemical assignments made by Kubanek and colleagues (Kubanek et al., 2003), and sedimentation velocity analysis confirmed the formation of an actin dimer ($s_{20,w}$ = 5.1) in solution upon addition of lobophorolide to monomeric G-actin (Figure S2). This complex appears to be stabilized by lobophorolide in two ways: 1) the macrolactone ring of each lobophorolide molecule is oriented so that each actin-lobophorolide unit presents a self-complementary hydrophobic surface, creating a 300 Å^2 dimerization interface that is stabilized by the hydrophobic effect and van der Waals contacts (Figure 1C), and 2) each lobophorolide molecule interacts with both actin subunits to help tether the complex together and bury a combined 2603 Å^2 of molecular surface area on the actin subunits.

No other monomeric macrolides are known to form such a quaternary complex; however, the actin-lobophorolide complex is strikingly similar to the 2:1 actin-swinholide A and actin-rhizopodin complexes, with the exception that the orientation of their actin subunits differ by a twist angle of approximately 18° and −22°, respectively (Figure S3; Hagelueken et al., 2009; Klenchin et al., 2005). A global alignment of both actin subunits for the lobophorolide and swinholide complexes provides a view of the extensive similarities in the three-dimensional space occupied by analogous atoms of each macrolide (Figure 2A). It also reveals that the interface between the two lobophorolide molecules occupies the same position as the site where the macrocycle of swinholide crisscrosses to produce the figure-eight-like conformation that allows both of its side chains to interact with the two actin molecules. Analogously to swinholide, the symmetrical arrangement of the actin subunits bound to lobophorolide is incompatible with the lateral arrangement of actin subunits between protofilaments in models of the F-actin double helix (Holmes et al., 1990), and F-actin nucleation complexes (Reutzel et al., 2004). These commonalities highlight the importance of the ring stacking interaction in formation of the lobophorolide-actin complex and lend support to the functional relevance of the unusual binding stoichiometry observed. However, the covalent connection between the two halves of swinholide likely creates a more stable and more rapidly assembled actin-macrolide complex than that mediated by lobophorolide, which may explain the less complete conversion of monomer to dimer by lobophorolide