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Structure of Bistramide A−Actin Complex at a 1.35 Å Resolution

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Bistramide A (**1**) is a marine natural product isolated from the ascidian *Lissoclinum bistratum*.[1−3] In addition to a highly potent antiproliferative activity, bistramide A altered the voltage dependence of the muscle twitch tension and inhibited Na^+ conductance.[4,5] We have recently developed an efficient and fully stereocontrolled synthesis of bistramide A, which established the structure of this natural product.[6,7] Furthermore, we revised a previously suggested mode of action[8] and identified actin as a specific cellular receptor of bistramide A,[9] which provided the molecular basis for its potent antiproliferative profile. This finding was enabled by a series of cell-based and in vitro studies coupled with affinity-based protein isolation using a fully synthetic bistramide A−biotin conjugate.[9] Importantly, bistramide A does not bear any structural resemblance with any of the known G-actin-binding natural products.[10] Thus, it was our intention to understand the basis of molecular recognition of actin by the bistramides. Herein, we report the results of the X-ray structure determination of actin−bistramide A complex, which unveiled the unique mode of binding of this natural product, enabling subsequent rational design of useful chemical probes for studies of actin cytoskeleton and development of potential leads for a new class of anticancer agents.[11,12]

Crystals of actin−bistramide A diffracted to up to 1.3 Å resolution. The structure was solved by molecular replacement and refined to a resolution of 1.35 Å. The structure of the complex is shown in Figure 1. Actin is found in a typical closed conformation[13] bound to ATP between subdomains 2 and 4 (Figure 1B) and 4 Ca^{2+} ions (not shown).[14,15] Bistramide A binds to actin between subdomains 1 and 3 (Figure 1B and 1C). A similar binding region of the protein is targeted by actin-capping protein gelsolin[16] and several actin-binding natural products, including kabiramide A,[17] swinholide A,[18] as well as structurally related macrolides.[19] The binding mode of bistramide A, however, has several highly unique features. In contrast to kabiramide A and swinholide A, bistramide A spans the entire deep binding cleft between subdomains 1 and 3 in its fully elongated conformation (ca. 2.5 nm), forming a network of extensive hydrogen-bonding contacts (vide infra). Upon binding, 64% (712 $Å^2$) of the solvent-accessible surface area of bistramide A is sequestered by the interaction with actin, providing the physical basis for the high binding affinity of the natural product to its receptor (K_d = 7 nM).[9]

The electron density of bistramide A is shown in Figure 2A. While the C(19)−C(40) spiroketal and the C(14)−C(18) amino acid subunits are highly ordered, the C(1)−C(4) enone side chain attached to the pyran fragment is disordered, suggesting that this moiety does not play a critical role in the binding of the natural product. Indeed, the enone is oriented out of the binding site and pointed toward the solvent. The known cell-based structure−activity relationship of bistramides is fully consistent with this observation. Indeed, structural changes of the C(1)−C(4) enone subunit in bistramides B and D do not alter significantly the cell growth inhibitory activities of these compounds. This finding further explains why our biotin−bistramide A conjugate, which was constructed by extension of the enone moiety, was used successfully for affinity-based actin isolation.[9]

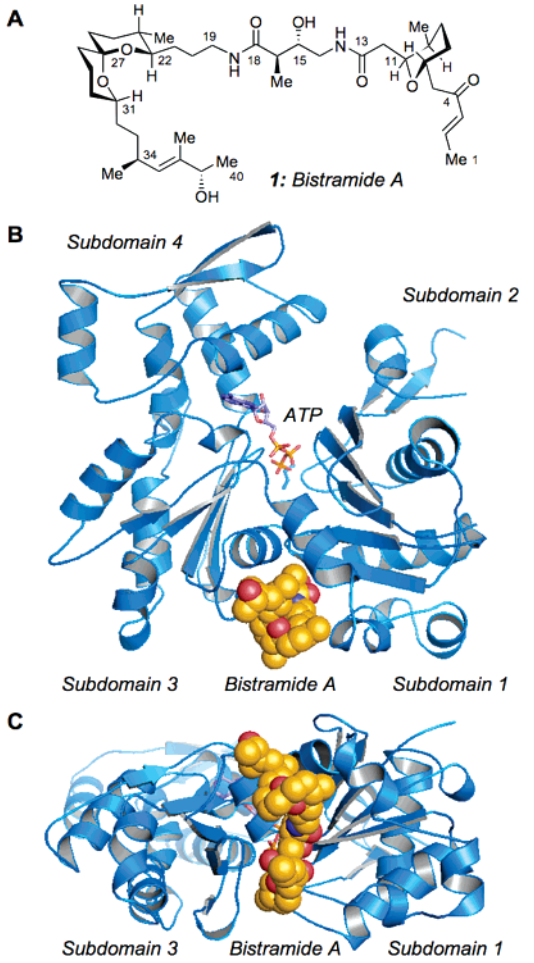

Figure 1. Structures of bistramide A and actin−bistramide A complex. (A) The structure of bistramide A is composed of three subunits, including C(19)−C(40) spiroketal, C(14)−C(18) amino acid, and C(1)−C(13) pyran. (B) The structure of actin−bistramide A complex depicting four actin subdomains, ATP, and bistramide A. (C) The structure of actin−bistramide A complex in a different orientation depicting bistramide A bound to the cleft between subdomains 1 and 3.

The most notable aspect of the bistramide A−actin structure is an extensive hydrogen-bonding network established upon a deep penetration of the central C(13)−C(18) amino acid-containing segment of bistramide A into the actin-binding pocket. It is noteworthy that swinholide A, kabiramide A, and related macrolides display very few polar contacts upon binding to actin with extensive hydrophobic interactions playing the dominant role. In the case of

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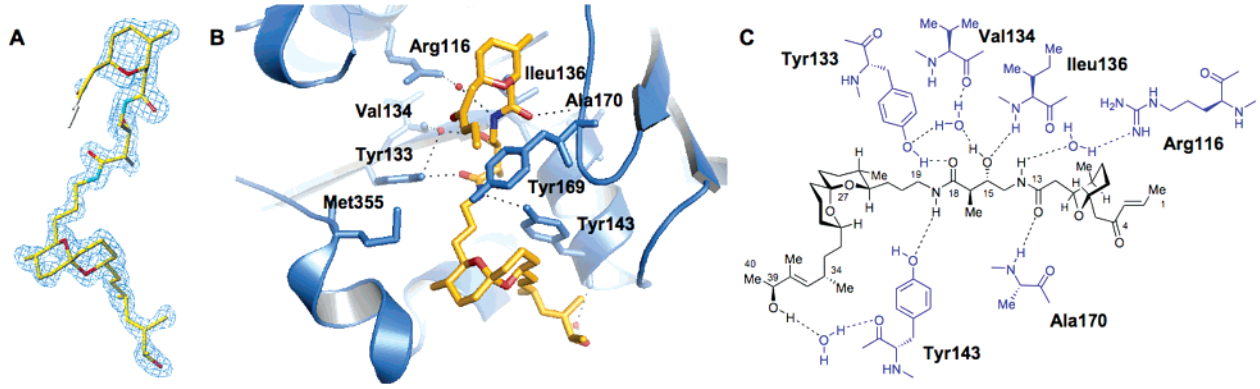

Figure 2. Electron density map of bistramide A and selected contacts involved in bistramide A−actin recognition. (A) The SigmaA-weighted $2F_o-F_c$ electron density map of bistramide A. (B) Selected amino acid side chains interacting with bistramide A. (C) Polar contacts between bistramide A and actin.

bistramide A, however, the five heteroatoms of the central fragment are involved in hydrogen-bonding interactions with Tyr 133, Val134, Ileu136, Arg116, Tyr143, and Ala170 (Figure 2B and C). Notably, four of six polar contacts are direct hydrogen-bonding interactions between the ligand and the protein. In addition, C(39) alcohol generates contacts with Tyr133 via a bridging water molecule. It is also highly noteworthy that the side chains of Tyr169 and Met355 are located directly over the bound small-molecule ligand, creating a deep, channel-like binding site. We propose that the polar contacts play the dominant role in the binding of bistramide A to actin. Indeed, the C(13)−C(18) central amino acid subunit is conserved in all bistramide congeners.

The C(19)−C(40) spiroketal subunit of bistramide A occupies a large hydrophobic pocket formed by Tyr143, Thr351, Ilue345, Thr148, Gly168, Leu349, and Leu346. The C(1)−C(13) pyran fragment generates very few contacts and is most likely not critical for binding. We propose that the spiroketal and the pyran subunits are important in attenuating the lipophilicity of bistramide A, favoring the docking of the natural product into the actin-binding pocket. *This structural information combined with our ability to chemically modify the bistramide framework*[6] *provides the basis for rational development of a series of new synthetic analogues as useful probes for studying actin cytoskeleton and as potential therapeutic leads.*

In summary, we have determined the structural basis of recognition of G-actin by bistramide A, which uniquely features an extensive network of hydrogen-bonding contacts combined with a series of hydrophobic interactions. The natural product binds in a deep cleft between subdomains 1 and 3, spanning the entire width of the protein. The same binding region has been implicated in forming contacts with the complimentary residues of the neighboring actin subunit in F-actin.[20] While the exact mechanism of F-actin depolymerization by bistramide A has not been elucidated, the structure of bistramide A−actin complex provides the first insight into the observed ability of the natural product to modulate G-actin polymerization by occupying the binding site, which is required for this process. The structure also suggests that bistramide A is expected to cap the filamentous actin at the barbed end.[16,17] Furthermore, bistramide A stabilizes the closed actin conformation and should inhibit nucleotide exchange. Experiments to test these predictions are in progress and results will be reported in due course.

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Supporting Information Available: Crystallization, data collection, and refinement details. Coordinates and structure factors have been deposited in the Research Collaboratory for Structural Biology (http://www.rcsb.org); PDB ID code is 2FXU. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Gouiffès, D.; Moreau, S.; Helbecque, N.; Bernier, J. L.; Henichart, J. P.; Barbin, Y.; Laurent, D.; Verbist, J. F. *Tetrahedron* **1988**, *44*, 451−459.

(2) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1354−1359.

(3) Biard, J. F.; Roussakis, C.; Kornprobst, J. M.; Gouffes-Barbin, D.; Verbist, J. F. *J. Nat. Prod.* **1994**, *57*, 1336−1345.

(4) Sauviat, M. P.; Verbist, J. F. *Gen. Phys. Biophys.* **1993**, *12*, 465−471.

(5) Sauviat, M. P.; Gouiffes-Barbin, D.; Ecault, E.; Verbist, J. F. *Biochim. Biophys. Acta* **1992**, *1103*, 109−114.

(6) Statsuk, A. V.; Liu, D.; Kozmin, S. A. *J. Am. Chem. Soc.* **2004**, *126*, 9546−9547.

(7) For structure elucidation and synthesis of bistramide C, see: (a) Wipf, P.; Uto, Y.; Yoshimura, S. *Chem.−Eur. J.* **2002**, *8*, 1670−1681. (b) Wipf, P.; Hopkins, T. D. *Chem. Commun.* **2005**, 3421−3423.

(8) Griffiths, G.; Garrone, B.; Deacon, E.; Owen, P.; Pongracz, J.; Mead, G.; Bradwell, A.; Watters, D.; Lord, *J. Biochem. Biophys. Res. Commun.* **1996**, *222*, 802−808.

(9) Statsuk, A. V.; Bai, R.; Baryza, J. L.; Verma, V. A.; Hamel, E.; Wender, P. A.; Kozmin, S. A. *Nat. Chem. Biol.* **2005**, *1*, 383−388.

(10) Yeung, K. S.; Paterson, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 4632−4653.

(11) Riou, D.; Roussakis, C.; Biard, J. F.; Verbist, J. F. *Anticancer Res.* **1993**, *13*, 2331−2334.

(12) Giganti, A.; Friederich, E. *Prog. Cell Cycle Res.* **2003**, *5*, 511−525.

(13) For a more detailed description of the protein conformation, see Supporting Information.

(14) Kablsch, W.; Mannherz, H. G.; Suc, D.; Pai, E. F.; Holmes, K. C. *Nature* **1990**, *347*, 37−44.

(15) Otterbein, L. R.; Graceffa, P.; Dominguez, R. *Science* **2001**, *293*, 708−709.

(16) McLaughlin, P. J.; Gooch, J. T.; Mannherz, H.-G.; Weeds, A. G. *Nature* **1993**, *364*, 685−692.

(17) (a) Klenchin, V. A.; Allingham, J. S.; King, R.; Tanaka, J.; Marriott, G.; Rayment, I. *Nat. Struct. Biol.* **2003**, *10*, 1058−1063.

(18) Klenchin, V. A.; King, R.; Tanaka, J.; Marriott, G.; Rayment, I. *Chem. Biol.* **2005**, *12*, 287−291.

(19) (a) Allingham, J. S.; Tanaka, J.; Marriott, G.; Rayment, I. *Org. Lett.* **2004**, *6*, 597−599. (b) Allingham, J. S.; Zampella, A.; D'Auria, M. V.; Rayment, I. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 14527−14532.

(20) Holmes, K. C.; Popp, D.; Gebhard, W.; Kabsch, W. *Nature* **1990**, *347*, 44−49.

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