Absolute Stereochemistry of Ulapualide A

John S. Allingham, Junichi Tanaka, Gerard Marriott, and Ivan Rayment

Supporting Information

Methods

Toxins isolation. Ulapualide A (**1**) was isolated from *Hexabranhus sanguineus* eggs collected at Pupukea, O'ahu, and placed in MeOH. Partitioning with hexane and then carbon tetrachloride, followed by HPLC of the carbon tetrachloride residue to give ulapualide A as a colorless oil. The identity of ulapualide was confirmed by NMR and mass spectroscopy. Mycalolide A (**5**) was isolated from an unidentified orange sponge that was collected in the Goto Islands, Nagasaki, in 1994. The sponge (138 g) was extracted with Acetone-MeOH, and its lipophilic extract (0.72 g) was obtained. The extract was separated by Sephadex LH20, repeated silica gel column and reversed phase HPLC to give mycalolide A (1.0 mg) and B (2.1 mg).

Actin purification. Actin was purified from rabbit muscle acetone powder either according to the standard protocol¹ or by extraction with 0.4 M ammonium thiocyanate followed by chromatography on hydroxylapatite (D. Smith, R.W. Smith, I.R., unpublished). Freshly prepared actin at 2 mg/ml in G-buffer (2 mM Tris, 0.2 mM ATP, 0.2 mM CaCl₂, 1 mM NaN₃, pH 8.0) was mixed with 1.1 - 1.2 molar excess of ulapualide A on ice and concentrated by ultrafiltration to 10-12 mg/mL. The protein complex was frozen as 30 µl droplets in liquid nitrogen and stored at -80 °C.

Crystallization and data collection. Crystals of the actin-ulpualide A complex were grown by small-scale batch² by carefully mixing 5 μ L of the complex, in a 1:1 ratio with 100 mM MES pH 6.0, 15% methyl ether poly(ethylene glycol) 5000, 75 mM CaCl₂, 1 mM sodium azide, 1 mM TCEP. The mixtures were nucleated immediately by streak-seeding from an earlier crystallization screen. Crystals grew as thin plates of dimensions $\sim 0.3 \times 0.1 \times 0.02$ mm over 2-3 days at 20° C. The crystals were cryopreserved by transferring into the precipitant solution followed by a 10 minute equilibration in cryoprotectant solution containing 100 mM MES pH 6.0, 15% methyl ether poly(ethylene glycol) 5000, 100 mM CaCl₂, 20% ethylene glycol and flash frozen in a stream of cold liquid nitrogen gas. Data was collected at the SBC 19-ID beamline, Advanced Photon Source in Argonne, IL as 200 frames with 1.0° oscillations at a wavelength of 0.964 Å.

Data processing, structure solution and refinement. Diffraction data were integrated and scaled with the program HKL2000³. The structure was solved by molecular replacement with Molrep⁴ starting from the actin-jaspisamide structure (PDB accession code 1QZ6,⁵). The structure was refined with Refmac⁶. Water molecules were added to the coordinate set with ARP/wARP⁷ with subsequent manual verification.

Figure preparation. Figures were prepared with the program Pymol 0.92.⁸

References:

- (1) Spudich, J. A.; Watt, S. *J Biol Chem* **1971**, *246*, 4866-71.
- (2) Rayment, I. *Structure (Camb)* **2002**, *10*, 147-51.
- (3) Otwinowski, Z.; Minor, W. *Methods in Enzymology* **1997**, *276*, 307-326.
- (4) Vagin, A.; Teplyakov, A. *Acta Crystallogr D Biol Crystallogr* **2000**, *56*, 1622-4.
- (5) Klenchin, V. A.; Allingham, J. S.; King, R.; Tanaka, J.; Marriott, G.; Rayment, I. *Nat Struct Biol* **2003**, *10*, 1058-63.
- (6) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. *Acta Crystallogr D Biol Crystallogr* **1997**, *53*, 240-255.
- (7) Perrakis, A.; Harkiolaki, M.; Wilson, K. S.; Lamzin, V. S. *Acta Crystallogr D Biol Crystallogr* **2001**, *57*, 1445-50.
- (8) DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. <http://www.pymol.org>.