Structural Study of Actin–Aplyronine A–Tubulin Heterotrimeric Complex and Development of Actin-affinity Tags

([1]*Graduate School of Bioagricultural Sciences,* [2]*Graduate School of Pharmaceutical Sciences, and* [3]*Graduate School of Science, Nagoya University,* [4]*Degree Program of Pure and Applied Sciences, University of Tsukuba*)
○Didik Huswo Utomo[1], Akari Fujieda[1], Atsunori Oshima[2], Akihiro Narita[3], Hideo Kigoshi[4], Masaki Kita[1]
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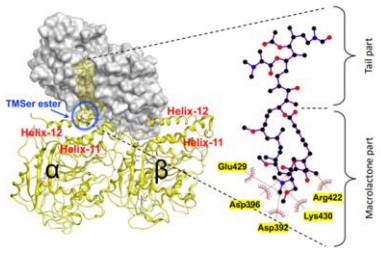
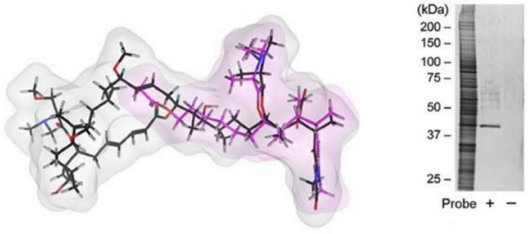
Aplyronine A (ApA) is a potent protein–protein interaction (PPI) inducer for actin and tubulin[1]. However, the binding position and antitumor molecular mechanism of ApA are still unclear. This study aimed to evaluate the heterotrimeric structure of "actin-ApA-tubulin" using cryo-electron microscopy (EM) and protein-protein docking approach. Initially, binding position analysis was performed by direct interaction between paclitaxel-stabilized microtubules (MTs) and actin-ApA. Negative staining-EM showed that MTs were rapidly disrupted by actin-ApA, but the binding position of actin-ApA on MT surface was unclear by cryo-EM analysis. To get heterotrimeric complex, single-particle analysis by cryo-EM was also examined, in which tubulin heterodimer and actin-ApA were directly mixed.

Next, protein-protein docking study was performed using molecular operating environment (MOE-2019) based on blind docking approach. 100 binding pose candidates were then screened based on the Gibbs free energy to get 10 heterotrimeric complexes. Further molecular dynamics simulation afforded a plausible heterotrimeric complex model, in which the C7 *N*,*N*,*O*-trimethylserine (TMSer) ester directly interacted with helix-11 and helix-12 of tubulin, being located at the outer surface of MT. These results suggested that the TMSer ester of ApA had an important role to stabilize the PPI between actin and tubulin heterodimer.

Inspired by ApA as a PPI inducer, we developed structurally-simplified C29–C34 side-chain analogs. The analogs possessing the C23 acyloxy group, the C29 *N,N*-dimethyl-L-alanine ester and the C34 *N*-methyl enamide selectively bound to actin and showed actin-depolymerizing activity, while reducing synthetic efforts.[2]



Heterotrimeric complex: actin-ApA-tubulin



Docking model of simplified analog and affinity purification

1) Kita, M. et al. *J. Am. Chem. Soc.* **2013**, *135*, 18089–18095; 2) Utomo, D. H. et al. *Chem. Commun.* **2021**, 57, 10540–10543.