

Structural insights into the phlorotannin binding of EHEP and akuBGL for producing biofuel from brown algae

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The world is meeting the challenge of the energy crisis, which prompted searching for renewable energy resource. Brown algae are ideal feedstocks for producing biofuels, the promising renewable resource. The sea hare *Aplysia kurodai* is an excellent model for investigating the biofuel production process. It consumes brown algae as a staple food to release a large amount of glucose from laminarin-depolymerization by the β -glucosidase in its digestive fluid (*akuBGL*). However, brown algae produced abundant secondary metabolite as a defense against the herbivores, such as phlorotannin. Phlorotannin inhibits *akuBGL* activity, which causes a problem in biofuel production from brown algae. Interestingly, Eisenia hydrolysis enhancing protein (EHEP) existed in the digestive fluid of *Aplysia kurodai* and, was found to protect *akuBGL* from phlorotannin-inhibition by binding with phlorotannin and precipitating¹. How EHEP bind to phlorotannin, why it can protect *akuBGL* from inhibition both are unknown.

In this study, we obtained EHEP and *akuBGL* from digestive fluid of *A. kurodai*. We determined the structures of EHEP in apo form and complex with an analogue of the phlorotannin, tannic acid by native-SAD method². The structures reveal that EHEP consisted of three chitin-binding domains linked by two long loops (Fig.1) and tannic acid bound at the center of EHEP. Furthermore, we determined the structure of *akuBGL* and showed it comprised of two GH1 (glycoside hydrolysis family 1) domains linked by the loop (Fig.2). Additionally, the docking analysis of tannic acid with *akuBGL* was performed, revealing the tannic acid occupies the active pocket of *akuBGL*. Collectively, we proposed the mechanism of EHEP that protects *akuBGL* from inhibition.

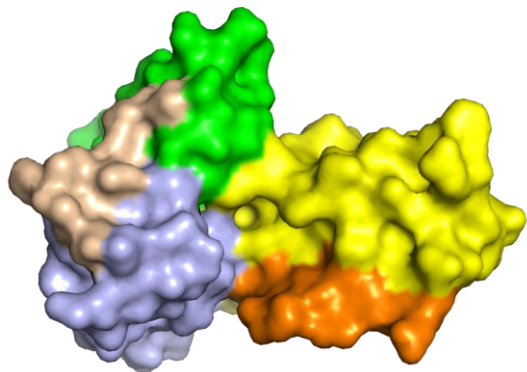


Figure 1. Complex structure of EHEP-tannic acid

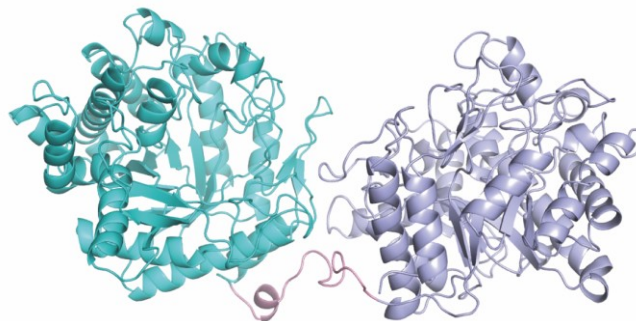


Figure 2. *akuBGL* structure

[1] Tsuji, A., Kuwamura, S., Shirai, A. & Yuasa, K. (2017). PLoS One 12, e0170669.

[2] Sun, X., Ye, Y., Sakurai, N., Kato, K., Yuasa, K., Tsuji, A. & Yao, M. (2020). Acta Crystallographica Section F-Structural Biology Communications 76, 20-24

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