学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称	博士（生命科学）	氏 名	孫 暁梅
Degree requested	Doctor of Life Science	Applicant name	SUN Xiaomei

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学 位 論 文 題 名
Title of Doctoral Dissertation

Characterization and ligand-binding manner of EHEP and BGL for producing biofuel from brown algae
（褐藻からバイオ燃料を生産するタンパク質 EHEP と BGL の特性およびリガンド結合様式の研究)

博士学位論文審査等の結果について（報告）

Dramatic increases in fuel demands globally have prompted a search for renewable energy sources. Biofuel is a promising alternative to fossil fuels because of its lower cost, renewable supply, and reduced greenhouse gas emissions. Brown algae are considered ideal feedstocks for producing biofuel because of their many advantages.

The sea hare *Aplysia kurodai* consumes brown algae as a staple food, using β-glucosidases (*aku*BGLs) as catalysts for hydrolyzing the glycosidic bonds of laminarin abundant in brown algae to produce glucose, making it an excellent model for investigating the biofuel production. However, phlorotannin which is also abundant in brown algae, inhibits the hydrolysis reaction of *aku*BGLs, bringing the serious problem in the production of biofuel source glucose from brown algae. Interestingly, Eisenia hydrolysis enhancing protein (EHEP) in the digestive fluid of *A. kurodai*, was found recently to protect *aku*BGL from this inhibition by binding and then precipitating with phlorotannin. An understanding of phlorotannin-binding manner with EHEP and *aku*BGL will lead us to elucidate protective mechanisms of EHEP and the phlorotannin-inhibitory mechanism of akuBGL. The knowledge could provide information on

how to increase the activity of akuBGL and how EHEP can be used recycled and the three-dimensional structures of akuBGL and EHEP are indispensable.

To understand the TNA-binding manner of EHEP, we tried to analyze the structure of EHEP firstly. Because EHEP is a novel and unique protein, with no structures of homologous proteins, were reported, we determined the structure of EHEP by single-wavelength anomalous dispersion using sulfur atoms in the protein as scattering factors (native-SAD) using a long wavelength. We also attempted to get the complex structure of EHEP with tannic acid (TNA), an analog of phlorotannins by soaking apo crystal into TNA solution for one month. The structure of EHEP consists of three chitin-binding domains (ChBD1, 2, and 3) linked by two long loops. In the EHEP-TNA structure, TNA is located on the surface of EHEP, and outmost five gallic acids cannot be visualized due to structural disorder. TNA bound to EHEP by hydrogen bond and hydrophobic interactions, and the TNA-binding did not induce large conformation changes of EHEP. Based on structural information, we consider that precipitation of EHEP with TNA should be recovered and successfully dissolve the precipitate of EHEP-TNA in alkaline pH.

Furthermore, we determined the structure of akuBGL which consists of two GH1 domains (D1 and D2) and they adopt almost the same structure with a rmsd of 0.59 Å for 371 Cα atoms (40.5% sequence identity). This is the first time to visualize the structure that contains two GH1 domains of BGLs. Like other structures of GH1 family enzymes, each domain exhibits a classical $(\beta/\alpha)_8$-barrel fold. D2 processes an active site containing two conserved carboxylic acid residues (E675, E885), while the first catalytic residue in D1 was mutated to D192. The two-domain architecture might represent a specific adaption towards brown algae. Moreover, docking was performed to analyze the inhibition mechanism of tannic acid with *aku*BGL. Taken all results together, we proposed the inhabitation and protection mechanism.

In conclusion, in this study, the applicant has new findings on phlorotannins binding manner of EHEP and *ana*BGL, which does not only contribute important information to life science and provide also molecular bases for designing new enzymes to produce biofuel from brown algae. In addition, through this study, the applicant has acquired a scientific intellectual and acting power.

Therefore, we acknowledge that the applicant is qualified to be granted the Doctorate of Life Science from Hokkaido University.