Functional characterization of vanadium-dependent bromoperoxidase from the red alga *Laurencia saitoi*

（紅藻マギレソゾ *Laurencia saitoi* 由来バナジウム依存型ブロモペルオキシダーゼの特性解析）

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Introduction

Various brominated compounds have been reported from the red alga genus *Laurencia*. Especially from *Laurencia saitoi*, many brominated triterpene compounds, such as thyrsiferol and magireols were reported to have good cytotoxicity and antifouling activity. The bromine addition biosynthesis reaction is presumed to be catalyzed by vanadium-dependent bromoperoxidase (VBPO). Two types of VBPO genes (*LsVBPO1* and *LsVBPO2*) have been cloned from *L. saitoi*. And *Ls*VBPOs were successfully expressed by recombinant *E. coli* in the previous research. The optimal pH dependence, vanadate requirement, and halogen selectivity of *Ls*VBPO2 have been reported. Moreover, a synthetic product of (6*S*, 7*S*, 10*R*, 11*R*, 14*R*, 15*R*, 18*S*, 19*S*) -squalene tetraepoxide, which is a putative precursor of brominated triterpenoids, was subjected to reaction with *Ls*VBPO2. However, the yield of brominated products was not enough to determine structure. This research aims to determinate the function of *Ls*VBPOs in triterpenoid biosynthesis by determination of *Ls*VBPO reaction products.

Methods

Recombinant *Ls*VBPO2 produced using an *E. coli* expression system was purified by ammonium sulfate precipitation and anion exchange chromatography. The concentration and activity of the purified enzyme was examined in an assay using MCD (monochromodimedone) and BCA (Bicinchoninic Acid) Protein Assay. To increase the amount of brominated product, reaction condition was optimized by LC-MS analysis. After multiplied small-scale brominated reactions, the brominated products were purified using HPLC. The gross structure of products was investigated by MS ,^{1}H and ^{13}C NMR.

Results and Discussion

The temperature of expression induction was changed from 25 °C to 16 °C. The reaction time was changed from 18 h to 30 min. As a result the yield of monobromide was increased by 30 times to reach 30%. Since large-scale reaction was failed, the small-scale reaction strategy was repeated to multiple times. Finally, 2.8 mg of monobromide ($C_{30}H_{49}BrO_6$) was obtained. Its gross structure was predicted by ^{13}C and ^{1}H NMR. However, the structure is quite different from the magireols, and no tetrahydropyran structure was found. The monobromide contained half of the squalene tetraepoxide structure. Under the excessive *Ls*VBPO2 (4 μg), the monobromide will become to a dibromide ($C_{30}H_{50}Br_2O_7$), both of them are unnatural compound.

In conclusion, after improving the reaction conditions in this study, we obtained enough monobromide for NMR analysis. But the results showed that the monobromide was not either magireols or the other natural triterpenoids. *Ls*VBPO2 catalyzed the bromination reaction, but its cyclization ability has not been found so far. In order to verify the biosynthetic pathway hypothesis, other enzymes or other reaction conditions are needed to simulate reactions in organisms.