

ABSTRACTS (MASTER THESIS)

Crystallization of hinokiresinol synthase

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Wood constitutes about 90% of terrestrial biomass. Wood is composed of sapwood and heartwood, the latter of which occupies large part of large-diametered wood and contains secondary metabolites called heartwood constituents. Heartwood constituents affect the coloration and physical property of heartwood such as vibration characteristic and dimensional stability. Furthermore, heartwood constituents usually have various biological activities. The presence of heartwood constituents may contribute to the longevity of trees. To elucidate the mechanism of heartwood formation is important to improve the quality of wood biomass.

Norlignan is a class of heartwood constituents typically found in conifers such Cupressaceae and Araucariaceae. Norlignans are also found in monocotyledonous species. Previous research revealed that norlignans, *cis*- and *trans*-hinokiresinols, were enzymatically formed from *p*-coumaroyl CoA and *p*-coumaryl alcohol. Later, the two genes (*HRS α* and *HRS β*) encoding *cis*-hinokiresinol synthase (*HRS*) were identified. Interestingly, recombinant proteins obtained by individual expression of *HRS α* and *HRS β* catalyzed the formation of (7*S*)-*trans*-hinokiresinol at 20.6 and 9.0% enantiomer excess (% e.e.). By contrast, the equivalent mixture of recombinant *HRS α* and *HRS β* catalyzed the formation of only (7*S*)-*cis*-hinokiresinol with more than 99% e.e. These results indicated that the subunit composition of *HRS* is able to control *cis/trans* isomerism and enantioselectivity in hinokiresinol formation (Figure). However, the reaction mechanism mediated by *HRS* and the stereochemical regulatory mechanism of the reaction by subunit composition have not been elucidated. As a first step towards the elucidation of these mechanisms, we established crystallization condition of recombinant *HRS β* .

The author established a large-scaled purification of recombinant *HRS*s by the combination of immobilized metal ion affinity chromatography (IMAC) and anionic exchange chromatography. Yield of purified *HRS*s was about 0.5 mg L⁻¹ culture medium for recombinant *HRS α* and about 5 mg L⁻¹ culture medium for recombinant *HRS β* and a recombinant protein (*HRS $\alpha\beta$*) coexpressed with *HRS α* and *HRS β* . However, recombinant *HRS*s were found to be easily aggregated or denatured during storage in an unoptimized condition. Therefore, the optimized condition for the storage of *HRS*s was screened by thermal shift assay. As a result, the presence of polyols was found to stabilize recombinant *HRS β* and *HRS $\alpha\beta$* , respectively.

Using the stabilized condition for storage of recombinant *HRS*s, we screened a crystallization condition for recombinant *HRS*s. Among recombinant *HRS*s, recombinant *HRS β* was successfully crystallized at 293 K by the sitting-drop vapor-diffusion method. The crystals thus obtained were analyzed by X-ray diffraction experiments in SPring-8. X-ray diffraction was observed in two crystals. A crystal obtained in the reservoir solution supplemented with 0.01 mM *p*-coumaric acid diffracted to 3.85 Å resolution, and a crystal obtained in the reservoir solution supplemented with 0.1 mM *p*-coumaryl alcohol diffracted to 3.15 Å resolution. Further optimization of crystallization condition would be needed to obtain higher resolution of diffraction.

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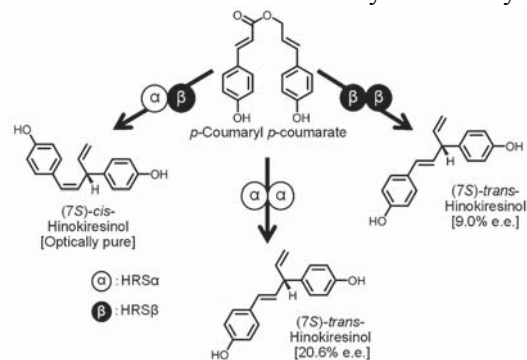


Figure. Hinokiresinol formation controlled by *HRS* subunit composition.