RECONSTRUCTION OF ANCESTRAL L-AMINO ACID OXIDASES TO BROADEN SUBSTRATE SELECTIVITY

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Characteristic functions of enzymes, such as high thermal stability and substrate specificity, are attained during the evolutionary process. Ancestral sequence reconstruction (ASR) is applied to infer the process by designing artificial enzymes which are located on ancestral node of phylogenetic tree; here, the inferred enzymes called ancestral enzymes. Ancestral enzymes often exhibit substrate promiscuity and high thermal stability of which functions are suitable to perform enzyme engineering. In addition, applicability of the ASR is high because the method requires only sequence data to design ancestral enzymes. Thus, we believe that artificial enzymes contributing to progress in enzyme engineering can be designed by ASR.

Success or failure of the ASR is strongly dependent on quality for multiple sequence alignment of sequence library which formed by sequences of target enzyme and their homologs. Thus, curation approach to generate optimal library is helpful to improve the succession rate. In this study, we tried to suggest the new approach through inferring of the evolutionary process of L-arginine oxidase (AROD), an FAD-dependent amino acid oxidase that exhibits high specificity toward L-arginine. Curation of AROD is difficult tasks because only one sequence data of native AROD is available for now; there is no structural and mutational data. Firstly, the library was prepared by selecting sequences that the 15th, 50th, 332nd and 580th residues are Gly, Ser, Trp and Thr, respectively. We omitted the sequences bearing extremely short or long and those with low sequence identity. The selection and exclusion of the sequences were performed by our original script. Finally, we can obtain three ancestral ARODs (AncARODn0, AncARODn1 and AncARODn2) using the library. In addition, we expressed the ancestral ARODs as well as native AROD (OkAROD) in bacteria. Phylogenetic tree analysis indicated that AncARODn0 is phylogenetically most remote from OkAROD whereas AncARODn2 is most similar to OkAROD (Figure 1).

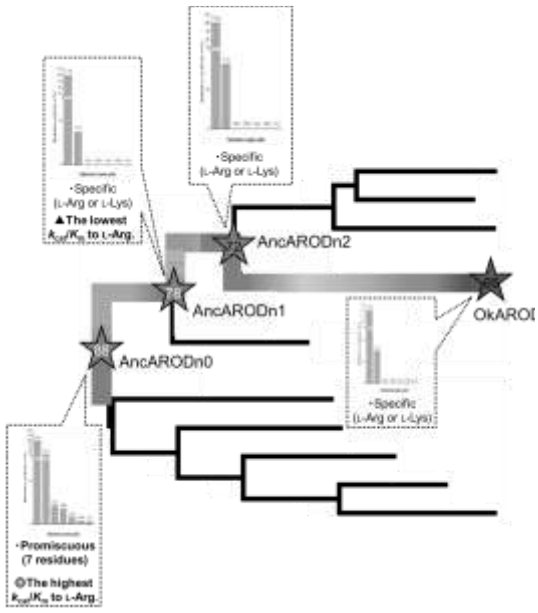

Figure 1. Schematic model representing biochemical properties of ancestral and native ARODs on a phylogenetic tree.

Biochemical analysis of the ARODs indicated that thermal stability was gradually increased by extending AROD sequences remote from native AROD. In fact, the $T_{1/2}$ values are following order: AncARODn0 (88 ºC) > AncARODn1 (78 ºC) > AncARODn2 (72 ºC) > OkAROD (65 ºC) (Figure 1). Remarkably, only AncARODn0 exhibits broad substrate selectivity similar to that of conventional promiscuous L-amino acid oxidase (LAAO) (Figure 1). Based on the results, we inferred that AROD may have evolved from a highly thermostable and promiscuous LAAO.

Furthermore, utilizing sequence data of AncARODn0 and identical procedure to design AncARODs, we succeeded in design of ancestral LAAO (AncLAAO) bearing broad substrate selectivity (> 10 of L-amino acids). AncLAAO can be expressed in soluble form utilizing BL21(DE3) expression system (>30 mg/L). AncLAAO can be used in deracemization of five DL-phenylalanine derivative with high enantio excess (> 99%, D-form) and conversion rate (> 76%). In this presentation, we will show the results of both AncAROD and AncLAAO.