TOPICS

Altering the Substrate Affinity of D-Lactate Dehydrogenase by the Molecular Evolution Method

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分子進化技術によるD乳酸合成酵素の効率化

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The effective catalytic properties of enzymes have been used in several industrial processes. Enzymes show high selectivity and reactivity under normal conditions but, in general, improved properties, such as activity, stability, selectivity and sensitivity are required. In order to endow enzymes with useful properties, research is moving from a "search for in nature" mode toward a "select tailor-made proteins from an artificial, diversified molecule library" mode. We have recently developed a new molecular evolution method called SIMPLEX (single-molecule-PCR-linked *in vitro* expression) for the purpose of altering protein function and stability[1] (**Fig. 1**).

In our recent studies, L-lactic acid (L-LA) was produced using metabolically engineered yeast that expresses exogenous L-lactate dehydrogenase.[2, 3] LA has both L- and D- enantiomers. Stereo complex formation between poly(L-lactic acid) and poly(D-lactic acid) is known to increase its melting point. Therefore, it is expected that the thermal stability of the stereo complex of poly lactic acid will be enhanced. In this study, we focus on improvement of the substrate affinity of D-lactate dehydrogenase (D-LDH) from *Leuconostoc mesenteroides* using SIMPLEX so as to achieve efficient production of D-LA.

Figure 2 shows a structural model of the active site of D-LDH. Amino acid residues, shown in light blue, are known to be pyruvate binding residues. Ala50 and Asn78, shown in yellow, which lie adjacent to pyruvate binding residues, and Ala234 and Asp260, shown in green, which lie adjacent to NAD (Nicotinamide adenine dinucleotide) ring, were selected and replaced with all kinds of amino acid residues. Approximately 10^4 of samples independently expressed on a 384-well plate were screened by measuring D-LDH activity in the presence of low-concentration pyruvate (0.05 mM), where the wild-type gave no detectable signal. The amino acid substitutions and the K_m values (Michaelis constants) for the pyruvate of the positive clones are shown in **Table 1**. All of the clones showed lower K_m values than that of the wild type. In particular, clone 2-3 utilized pyruvate slightly more efficiently, having a K_m of 0.12 mM. The pyruvate affinity of clone 2-3 was 2.3-fold higher than that of the wild type. Recent developments in protein engineering have provided important tools for the efficient development of new proteins.

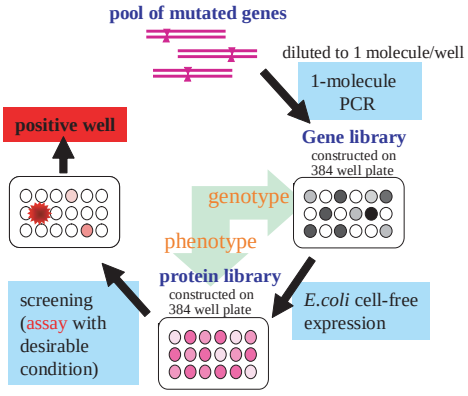

Fig. 1 The strategy of SIMPLEX.

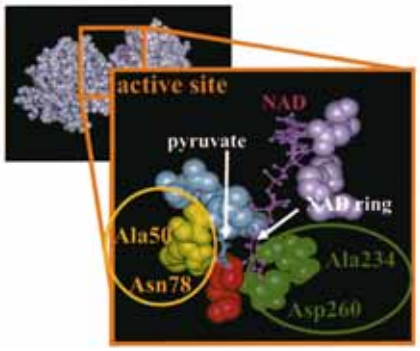

Fig. 2 The structural model of the active site of D-LDH.

Table 1 Result of the screening.

clone No.	amino acid No. pyruvate 50	78	NAD ring 234	260	*Km* (mM)
wild type	A	N	A	D	0.28
clone 1-1	A	S	A	D	0.23
clone 1-2	T	S	A	D	0.21
clone 2-1	A	N	T	D	0.20
clone 2-2	T	S	V	D	0.17
clone 2-3	A	S	T	D	0.12

References

1) Miyazaki-Imamura, C., et al. : Protein Eng., **16**(2003), 423
2) Ishida, N., et al. : Appl. Environ. Microbiol., (2005), in press.
3) Saitoh, S., et al. : Appl. Environ. Microbiol., (2005), in press.

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