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# Enzyme Evolution Using a Novel Cell Surface Display System in Yeast *Hansenula polymorpha*

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Directed evolution is one of the most effective methods currently available for protein engineering to get the desirable properties. Unlike protein engineering by rational design, directed evolution dose not require knowledge *a priori* of protein structure or structure-function relationship. High throughput screening tools, however, are absolutely required to avoid a labor-intensive procedure for the selection of a mutant with an improved property from the large pool of mutant library. Recently, cell surface display systems of bacteriophage, bacteria and yeast have drawn special attention not only for the screening of protein motifs from libraries for novel drug-targets, but also for the selection of mutant enzymes with desired properties after directed evolution for the novel biocatalysts.

For the development of a high throughput screening system of the functionally improved enzymes after directed evolution, a novel cell surface display system has been constructed in a yeast, *Hansenula polymorpha*. Several enzymes, such as lipase B of *Candida antarctica* (CalB), glucose oxidase (GOD) of *Aspergillus niger* and carboxymethylcellulase (CMCase) of *Bacillus subtilis* have been displayed on the cell surface using a novel glycosylphosphatidylinositol (GPI)-anchored cell wall protein, Cwp1p of *H. polymorpha*. All displayed enzyme activities could be detected on the whole cell fractions. To evaluate the surface display system for the selection of enzymes after directed evolution, several approaches have been made for the improvement of cell wall anchoring activity of Cwp1p, for the improvement of catalytic activities of CalB and GOD, respectively. Mutants of anchoring motif, Cwp1p, with 2-3 fold higher anchoring activity were easily screened by the selection of cell displaying higher amount of GOD on its surface using a powerful and high throughput screening tool, fluorescence activated cell sorter (FACS). For the improvement of catalytic activities of CalB, a cell surface displayed enzyme library was screened on halo-forming plates containing tributyrin. Two independent CalB mutants, CalB10 and 14, both showed around 5 times higher whole cell activities and 6-10 times higher activities as soluble secreted form. Sequence analyses of mutant *CALB* genes recovered from selected transformants revealed the amino acid substitution of $Leu^{278}Pro$ in CalB10 and $Leu^{278}Pro/Leu^{219}Gln$ in CalB14, respectively. As the substituting $Pro^{278}$ of both CalB mutants is located near the proline site at α10 helix, this mutation is assumed to induce conformational change in the α10 helix and increase the lipase activity. Site-directed mutagenized *CALB*, LQ ($Leu^{219}Gln$) was efficiently expressed and secreted around 3 folds

more into culture supernatant than wild type without increase of the transcript level. The GOD mutant with improved catalytic activity was also selected in similar way as CalB. Two GOD mutants, GOD3 and 6 with 2-3 fold higher activities compared with parental GOD showed the amino acid substitution of $Arg^{357}His$ in GOD3 and $Arg^{357}His/Pro^{545}Ser$ in GOD6, respectively.

In conclusion, our results demonstrate that a novel cell surface display system of *H. polymorpha* could provide a powerful screening tool for the evolution of various enzymes with improved properties.