外国語要旨

Preparation of recombinant human pancreatic lipase using *Escherichia coli* and the function of glycosylation of native pancreatic lipase

Nanami Kawaguchi

Background and Purpose

Human pancreatic lipase (HPL) is the main lipolytic enzyme involved in the digestion of dietary fat. Various eukaryotic expression systems have been employed for recombinant human pancreatic lipase (recHPL), such as those using yeast, insect, and mammalian cells [1-3]. However, *Escherichia coli* (*E.coli*), which is prokaryote and easily cultivated at low-cost, has not been used as an expression system for recHPL. Native HPL has an *N*-linked glycan on Asn^{167}, similar to porcine pancreatic lipase (PPL), but the role of the glycosylation has not been elucidated yet. In this study, a protocol for the preparation of active recHPL using *E.coli* was established for the first time, and the recHPL was characterized in various aspects including the activity. Furthermore, the role of glycosylation of pancreatic lipase was investigated.

Results and Discussion

1. Preparation of recHPL

The recHPL-ST II was solubilized using 8 M urea from *E.coli* lysate and purified on a Strep-Tactin Sepharose column. After refolding by stepwise dialysis for 2 days, followed by gel filtration, active recHPL was obtained. The SDS-PAGE profile of the purified protein under non-reducing conditions indicated that a part of refolded recHPL formed high-molecular weight aggregates, consisting of more than 4-5 molecules per aggregate, which formed through intermolecular disulfide bonds. Monomer recHPL showed lipolytic activity, while the aggregated form did not show degrading activity toward triglyceride.

2. Characterization of recHPL compared with native PPL

The molecular weights of recHPL and PPL were measured by MALDI-TOFMS. The measured masses for recHPL and PPL coincided with the theoretical values predicted from each primary sequence, and the glycan components reported for PPL [4]. The CD spectra of the recHPL and PPL indicated that they contain secondary structures of similar compositions, and using Ellman reagent,

both lipases were confirmed to contain one free thiol group. Lipolytic acticity, kinetic constants, pH dependency, and inhibitor sensitivity of the recHPL were equal to that of native PPL. These results indicate that the expression of recHPL using *E.coli* produces recHPL with equal activity and specificity to those of native pancreatic lipase.

3. **Role of glycosylation on pancreatic lipase**

The recHPL almost completely lost its lipolytic activity above 50 ^{o}C, showing a lower heat-stability than that of native PPL, which retained half of its activity at this temperature. Stability against proteolysis of the recHPL was lower than that of native PPL. These results indicate that the glycosylation on pancreatic lipase contributes to its molecular stability. Furthermore, several proteins which bind to the glycan of pancreatic lipase were found in intestinal brush border membrane (BBM). This result suggests that the glycan of pancreatic lipase may interact with ligands in the intestine, which may regulate the lipid degradation by the lipase and the product absorption.

Conclusion

An active recHPL was successfully prepared and characterized for the basic biochemical properties for the first time using the *E.coli* expression system. Compared with native PPL at 37 ^{o}C, recHPL prepared using this protocol showed almost equal specific activity, pH-dependency, substrate selectivity and inhibitor sensitivity. The recHPL may be used for the screening of inhibitor compounds against lipase, which may provide an important avenue for anti-obesity therapy. Lipases are one of the most widely used biocatalysts for biotechnological processes, and recHPL holds promise for many industrial applications. Our results also suggest that glycosylation of pancreatic lipase may partly contribute to the stabilization of its structure and lipolytic activity, and may play a role in the regulation of lipid digestion and absorption by binding to receptors in the intestine.

[1] Thirstrup, K., Carrière, F., Hjorth, S., Rasmussen, P. B., Wöldike, H., Nielsen, P. F., and Thim, L. (1993) *FEBS Lett* **327**, 79-84

[2] Canalias, F., Visvikis, A., Thioudellet, C., and Siest, G. (1994) *Clin Chem* **40**, 1251-1257

[3] Yang, Y., and Lowe, M. E. (1998) *Protein Expr Purif* **13**, 36-40

[4] Fournet, B., Leroy, Y., Montreuil, J., Děcaro, J., Rovery, M., van Kuik, J. A., and Vliegenthart, J. F. (1987) *Eur J Biochem* **170**, 369-371