(특강-4)

Protopanaxadiol Ginsenoside-Metabolizing Enzymes of Human Intestinal Bacteria

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Ginseng (the root of *Panax ginseng* C.A. Meyer, Araliaceae) is frequently used as a crude substance taken orally in China, Korea, Japan, and other Asian countries as a traditional medicine.[1)] The major components of ginseng are ginsenosides, glycosides containing an aglycone with a dammarane skeleton.[3,4)] These ginsenosides have been reported to show various biological activities including an increase of cholesterol metabolism, stimulation of serum protein synthesis, immunomodulatory effects, an antiinflammatory activity, and tumor effects (inhibition of tumor-induced angiogenesis and prevention of tumor invasion and metastasis).[5-7)] To explain these pharmacological actions, it is thought that ginseng saponins should be metabolized by human intestinal bacteria after they are swallowed.[8-12)] For example, ginsenoside Rb1, Rb2, and Rc are transformed to 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901 or compound K) by human intestinal bacteria. This IH-901 induces the anti-metastatic or anticarcinogenic effect by blocking tumor invasion or preventing chromosomal aberration and tumorigenesis.[13,14)] Related to the biotransformation of ginsenosides, Hasegawa *et al.* isolated *Prevotella oris* from human intestinal feces.[15)] However, the purification and characterization of the glycosidases of intestinal bacteria related to the metabolism of ginsenosides have not been studied.

Therefore, we isolated ginseng protopanaxadil ginsenoide-metabolizing intestinal bacteria, purified ginsenoside-metabolizing enzymes (β-glucosidase, α-L-arabinosidase, β–xylosidase and α-L-rhamnosidase) from human intestinal bacteria, and characterized their enzymes.

1) β-Glucosidase

From this *Fusodobacterium* K-60, a ginsenoside R_{b1}-metabolizing enzyme, β-glucosidase, has been purified. The enzyme was purified to apparent homogeneity by a combination of

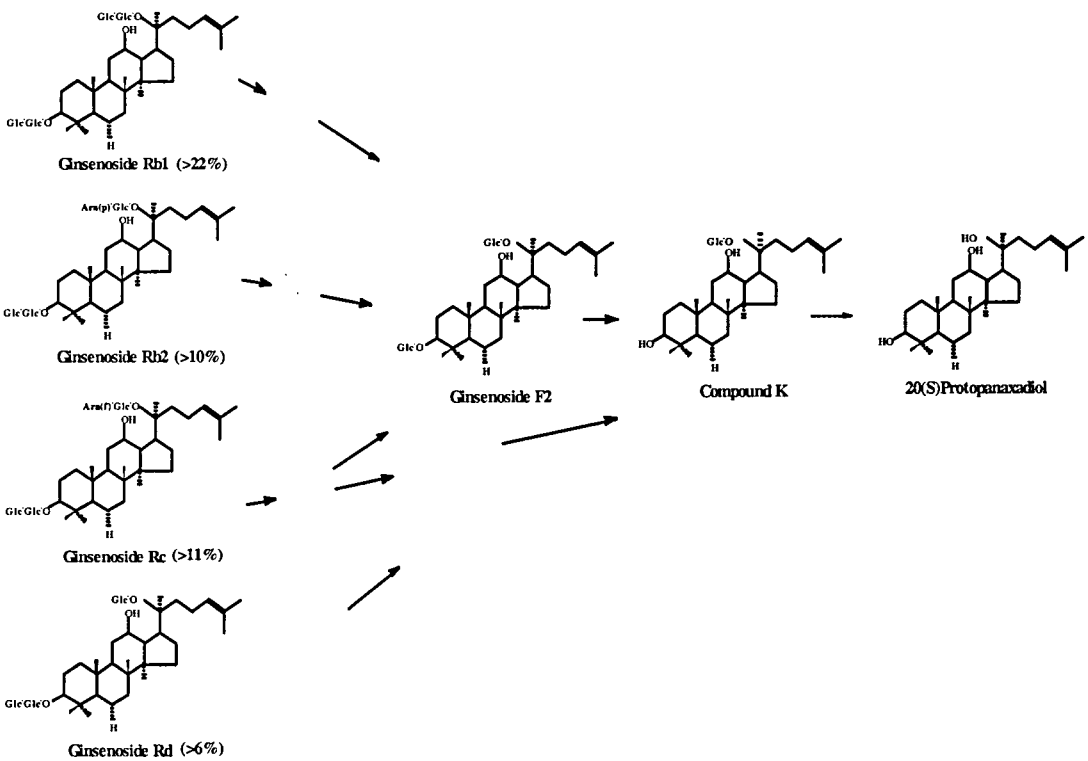
butyl-Toyopearl, hydroxyapatite ultragel, Q-Sepharose, and Sephacryl S-300 HR column chromatographies with a final specific activity of 1.52 μmol/min/mg. It had optimal activity at pH 7.0 and 40°C. The molecular mass of this purified enzyme was 320 kDa, with 4 identical subunits (80 kDa). The purified enzyme activity was inhibited by Ba^{++}, Fe^{++}, and some agents that modify cysteine residues. This enzyme strongly hydrolyzed sophorose, followed by p-nitrophenyl β-D-glucopyranoside, esculin, and ginsenoside R_{b1}. However, this enzyme did not change 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901) to 20(*S*)-protopanaxadiol, while it weakly changed ginsenoside R_{b1} to IH-901.

2) α-L-Arabinosidase

Two novel arabinosidases, α-L-arabinopyranosidase (no EC number) and α-L-arabinofuranosidase (EC 3.2.1.55), have been purified from ginsenoside-metabolizing *Bifidobacterium breve* K-110, which was isolated from human intestinal microflora. α-L-Arabinopyranosidase was purified to apparent homogeneity by a combination of DEAE-cellulose, butyl-toyopearl, hydroxyapatite, QAE-cellulose and Sephacryl S-300 column chromatographies with the final specific activity of 8.871 μmole/min/mg. α-L-Arabinofuranosidase was purified to apparent homogeneity by a combination of DEAE-cellulose, butyl-toyopearl, hydroxyapatite and Q-sepharose, Sephacryl S-300 column chromatographies with the final specific activity of 6.46 μmole/min/mg. Molecular weight of α-L-arabinopyranosidase is 310 kDa by gel filtration, which are consisted of four identical subunits (M.W. 77 KDa by SDS-PAGE), and that of α-L-arabinofuranosidase is 60 kDa by gel filtration and SDS-PAGE. α-L-Arabinopyranosidase and α-L-Arabinofuranosidase showed optimal activity at pH 5.5-6.0 and 40°C, and 4.5 and 45°C, respectively. Both purified enzymes were potently inhibited by Cu^{2+} and PCMS. α-L-Arabinopyranosidase acted to the greatest extent on p-nitrophenyl-α-L-arabinopyranoside, followed by ginsenoside Rb2. α-L-Arabinofuranosidase acted to the greatest extent on p-nitrophenyl-α-L-arabinofuranoside, followed by ginsenoside Rc. These enzymes did not act on p-nitrophenyl-β-galactopyranoside and p-nitrophenyl-β-D-fucopyranoside. These findings suggest that the biochemical properties and substrate specificities of these purified enzymes are different from those of the previously purified α-L arabinosidases and this is a first reported purification of α-L-arabinopyranosidase from an anaerobic *Bifidobacterium* sp.

3) β-D-Xylosidase

β-D-Xylosidase (EC 3.2.1.37) has been purified from ginsenoside Ra-metabolizing *Bifidobacterium breve* K-110, which was isolated from human intestinal microflora. β-D-Xylosidase was purified to apparent homogeneity by a combination of ammonium sulfate precipitation, QAE-cellulose, butyl-toyopearl, hydroxyapatit and Q-Sepharose column chromatographies with the final specific activity of 51.8 μmole/min/mg. Molecular weight of β-D-xylosidase is 49 kDa by SDS-PAGE and gel filtration, which is consisted of uni subunit. β-D-xylosidase showed optimal activity at pH 5.0 and 37°C. The purified enzyme was potently inhibited by PCMS. β-D-xylosidase acted to the greatest extent on *p*-nitrophenyl-β-D-xylopyranoside, followed by ginsenoside Ral, and ginsenoside Ra2. This enzyme hydrolzysed xylan to xylose, but did not act on *p*-nitrophenyl-β-glucopyranoside, *p*-nitrophenyl-β-galactopyranoside and *p*-nitrophenyl-β-D-fucopyranoside. These findings suggest that this is a first reported



Scheme 1. Proposed metabolic pathway of protopanaxadiol saponins by human intestinal bacteria

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