New concept of prebiotics : Prebiotic Enzyme Transglucosidase

Satoshi Koikeda

9th American Biotechnology Congress
What we are

Amano Enzyme Europe

Amano Enzyme China

Amano Enzyme Japan

Amano Enzyme USA

Amano Enzyme Mexico

Asia Pacific Office
World Markets for Enzymes (Estimated) 2015

Medical Use 2,430 (US $ Million)
- Diagnostics: 560
- Digestive Enzyme (including Supplement): 220
- Biotransformation: 230
- Enzyme Replacement Therapy: 710
- Thrombolysis: 710
- Other Research Use: 1,060
- Research & Biotechnology: 710

Food and Industrial Use 4,490
- Dairy: 425
- Baking: 625
- Brewing: 270
- Starch: 300
- Protein: 200
- Animal Feed: 545
- Fat & Oil: 30
- Protein: 200
- Protein: 200
- Fat & Oil: 30
- Leather: 15
- Waste Treatment: 25
- Pulp: 15
- Detergent: 1,080
- Bioethanol: 600
- Others: 50

Total 7,980

Reference: World Enzymes (Freedonia, 2011)
**Digestive aid enzyme has a long history**

*Aspergillus oryzae*

Invented by Jōkichi Takamine (1894)

Jōkichi Takamine, 1854 – 1922

Wheat bran

Water extraction
Powderization

Produced by Parke-Davis (1895)
Expected function of digestive aid enzyme

- Regulating Nutrition Absorption
- Regulating Nutrition absorption and Microbiota System

Novel digestive enzyme
Transglucosidase is bifunctional enzyme

Carbohydrate degradation

Nutrition

Prebiotics

Alpha Glucosidase

Transglucosidase reaction

Starch

Maltose

Glucose

Isomaltose

panose

Isomaltotriose

Starch degradation

Alpha Glucosidase
Prebiotic enzyme concept on Transglucosidase

Food Starch

Prebiotic Enzyme (Transglucosidase)  Digestive Enzyme

Oligosaccharide

Microflora  Slow digestion

Carbohydrate

Energy absorption

Bifidobacteria ↑  Lactobacillus ↑  Bacteroides ↑

Blood glucose↓

Weight management
**Transformed Oligosaccharides by Transglucosidase**

**In vitro analysis**

1. Stir and Dissolution with amylase
2. Adjust pH 5.5
3. Add TG 90ku
4. 37 °C, up to 90 min
5. HPLC Analysis

**Pasta (100g)**
- Carbohydrate 70g

**Noodle (100g)**
- Carbohydrate 70g

Graphs showing Oligosaccharide (g/meal) over time (0, 30, 60, 90 min) for both pasta and noodle samples.
Prevention of Postprandial Hyperglycemia and Hyperinsulinemia in Healthy Volunteer

- 522Kcal meal: Protein (14.4g), Fat (2.1g), Carbohydrate (111g) + Transglucosidase
- Blood sampling: every 30 min for 2 h

AUC of Blood Sugar (mg h/dL)

- 0 mg
- 150 mg
- 300 mg

AUC of Blood Insulin (µU h/mL)

- 0 mg
- 150 mg
- 300 mg

Efficacy in diabetes patients

Trial Design:
• 3 months’ randomized, double-blind, placebo-controlled trial

Inclusion criteria:
• Type 2 diabetes outpatient
• HbA1c is stable from 5.8% to 7.5% (JDS)  
  6.2% to 7.9% (NGSP)  
• Stable dosage of medication for at least 1 month
• Stable diabetes condition for at least 3 months  
  (change in HbA1c less than 1.0%)

Exclusion criterion:
• Gut resection history

**Study End Points**

Primary outcome:
• The change in HbA1c level

Secondary outcomes:
• The change in various other metabolic parameters
  Fasting blood glucose (FBG), Glycosylated albumin (GA),
  Plasma insulin level, Total cholesterol (T-CHO),
  High-density lipoprotein cholesterol (HDL-C),
  Low-density lipoprotein cholesterol (LDL-C),
  Triglyceride (TG), Free fatty acid (FFA),
  High-molecular-weight (HMW) adiponectin

* M. Sasaki et al. *Diabetes, Obesity and Metabolism*, 4, 379–382, 2012
Study flow diagram

Entrée (n=64)

Placebo (n=19)

- Withdrew from trial (n=3)
  - Stopped medication (n=1)
  - Deviation from protocol (n=2)

Placebo (n=16)

Transglucosidase, 300 mg/d (n=23)

- Withdrew from trial (n=5)
  - Stopped medication (n=1)
  - Left study because of hospitalization (n=1)
  - Lost to follow-up (n=1)
  - Deviation from protocol (n=2)

Transglucosidase, 300 mg/d (n=18)

Transglucosidase, 900 mg/d (n=22)

- Withdrew from trial (n=5)
  - Stopped medication (n=2)
  - Deviation from protocol (n=3)

Transglucosidase, 900 mg/d (n=17)
Primary outcome

Change of HbA1c

- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

***, p < 0.001 v.s 0M

-0.18% (p < 0.05)
-0.21% (p < 0.01)

Mean ± SE

Calculated by using a two-way ANOVA and Benferroni post-hoc test

Secondary outcomes (1)

**Fasting Blood Glucose**

- **Placebo**
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**Insulin**

- **Placebo**
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**Glycoalbumin**

- **Placebo**
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

Calculated by using a two-way ANOVA and Benferroni post-hoc test

- *, p < 0.05 v.s 0M
- **, p < 0.01 v.s 0M
- #, p < 0.05 v.s placebo
- ##, p < 0.01 v.s placebo

Secondary outcomes (2)

**BMI**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**Diastolic Blood Pressure**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**Adiponectin**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**ALT**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**AST**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d

**GGT**
- Placebo
- Transglucosidase, 300 mg/d
- Transglucosidase, 900 mg/d
Change of gut microbiota composition by transglucosidase administration

Placebo ➔ TG 300mg/day
TG 900mg/day

Bacteroidete/Firmicutes

Sasaki M et al. BMC Gastroenterology, 41, 2013


Weight loss
Possible action of prebiotic enzyme

Food starch

Transglucosidase

Oligosaccharide

Gut microbiota

Bifidobacteria ↑
Lactobacillus ↑

Slow digest ↓

Energy ↓

Bacteroidete/Firmicutes ↑

BMI ↓

SCFA ↑

• LDL-Cholesterol ↓
• Triglyceride ↓
• ALT, AST, GGT ↓
# Comparison between prebiotics and probiotics

<table>
<thead>
<tr>
<th>Prebiotics</th>
<th>Prebiotic Enzyme</th>
<th>Probiotics</th>
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</thead>
<tbody>
<tr>
<td>✓ Inconvenient intake</td>
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**Prebiotics**
- Inconvenient intake
- Unfamiliar food ingredient
- Safety in the case of overdose

**Prebiotic Enzyme**
- Convenient intake
- Natural diet

**Probiotics**
- Convenient intake
- Safety of living bacteria
Conclusin

- We proposed new enzyme concept: prebiotic enzyme.
- Developing Transglucosidase as prebiotic enzyme is on going.
- Clinical study resulted in lowering of HbA1c and blood insulin level, and improvements in metabolic and cardiovascular risk factors.
- Protein engineering makes Transglucosidase more efficiently.
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Production of Transformed Oligosaccharides by Transglucosidase

Corn Starch (DE 5～15) → IMOs (~30%)

58 °C
pH 5.5
48 hours
With amylase

Protein Engineering
**Amano Enzyme Group History**

1899  Pharmaceutical business started.

1948  Production of pharmaceutical enzyme (API) started.

1960  Enzyme R&D Center inaugurated.

1961  Production of food-processing enzyme started.

1970  Production of diagnostic enzyme started.

      It was transformed to current Amano Enzyme USA Co., Ltd. in Elgin IL.

1983  European office opened in Frankfurt, Germany. Now Amano Enzyme Europe Ltd. in Chipping Norton, OX, UK

2003  China office opened in Shanghai. Now Amano Enzyme China Ltd.

2012  Amano Enzyme Asia Pacific office opened in Kuala Lumpur, Malaysia.
Structure modeling of Transglucosidase

MOE program (Chemical Computing Group). The corrected aglA sequence from GenBank (accession number D45356) was aligned against Maltase-glucoamylase (Protein Data Bank: 2QLY).
Group 1 mutant

Group 1

WT

W343M

Substrate: 25% maltose

Maltose (α-1,4)

Isomaltose (α-1,6)

Maltotriose (α-1,4, α-1,4)

Panose (α-1,4, α-1,6)

α-1,4-linkages
Group 2 mutant

Group 2

Substrate: 25% isomaltose

α-1,6-linkages
Improving IMO production capability with protein engineering

Carbohydrase

α-glucosidase (yeast)
α-glucosidase (mammal)
Isomaltase (Bacteria)

Transglucosidase (original)

Group 1 mutants (W343X, S496X)
Group 2 mutants (S495X)

Transglycosylation

α-1,4

α-1,6