Unusual transglucosylation reaction on a soybean isoflavone by amylosucrase from two different bacterial sources

Eun-Ryoung Kim[1], Jung-Min Choi[1], Chan-Su Rha[1], Young-Sung Jung[1], Min-Ji Ko[1], Gi-Tae Kim[1], Dae-Ok Kim[1] and Cheon-Seok Park[1]

[1]*Graduate School of Biotechnology and Institute of Life Sciences & Resources, KyungHee University, Yongin 446-701 Korea*

ABSTRACT

Amylosucrase (ASase, EC 2.4.1.4) is belonging to glycoside hydrolase family 13 (GH13) and has uncommon three enzymatic activities of hydrolysis, polymerization and isomerization. ASase acts on substrates containing α-glucoside linkages and has a catalytic mechanism named α-retaining double displacement forming glycosyl-enzyme intermediate. In this study, two kinds of recombinant ASases from Neisseria polysaccharea (NPAS) and Deinococcus geothermalis (DGAS) were used for transglucosylation on daidzin. Daidzin is known as a major isoflavone and found in Kudzu and soybean. Transglucosylation of daidzin is expected to improve water-soluble, stability of chemical structure and taste. Two similar enzymes (NPAS and DGAS) showed quite different reaction products with same substrates. While the main transglycosylation products of NPAS reaction were daidzein-7-O-β-diglucoside and daidzein-7-O-β-triglucoside, those obtained by DGAS were daidzein-7-O-β-diglucoside and daidzin isomer. These results indicated that two enzymes may have different structural spaces inside of the catalytic sites, which leading to the uncommon transglycosylation reactions.

RESEARCH OBJECTIVES

- Expression and purification of recombinant DGAS and NPAS
- The comparison of bioconversion aspects both DGAS and NPAS
- The observation of influences according to the different reaction conditions
- The transglucosylation of daidzin with sucrose by DGAS and NPAS

INTRODUCTION

- Although daidzin, a major isoflavone, has many beneficial effects, its application is limited because of the several drawbacks. In this study, enzymatic modification is employed to broaden its applications.
- Amylosucrase has three enzymatic activities of hydrolysis, polymerization and isomerization. When sucrose is used as a sole substrate, ASase proceeds three-step catalytic reactions (Fig.1).
- The DGAS amino acid sequence exhibited 58.69 % identity with NPAS.

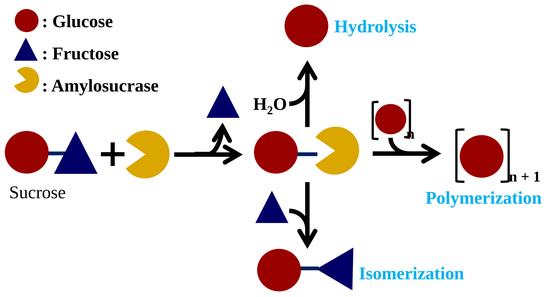


Figure 1. Action modes of ASase in hydrolysis, polymerization and isomerization of sucrose with water or acceptor molecule

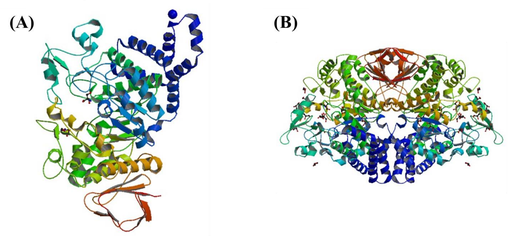


Figure 2. Crystal structure of amylosucrase from *Neisseria polysaccharea* (A) and *Deinococcus geothermalis* (B)

METHODS

Experimental methods

Expression cloning

Table 1. The constitutive expression systems for NPAS and DGAS

	NPAS	DGAS
Host	*Escherichia coli* BL21 (DE3)	*Escherichia coli* MC1061
Expression vector	pET-21a(+) (Novagen, Madison, WI, USA)	pHCXHD modified from pHCEII-*Nde*I (BioLeaders Co., Daejeon, Korea)
Induction conditions	1 mM Isopropyl-β-D-thiogalactopyranoside (IPTG), 18℃ for 18hrs	37℃ for 18hrs

Purification

- Ni-NTA affinity chromatography
- Buffer
  a. Lysis buffer
  b. Washing buffer
  c. Elution buffer

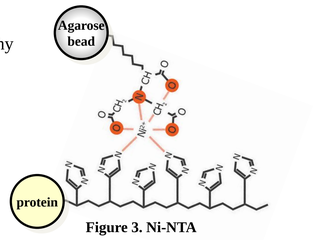


Figure 3. Ni-NTA

Reaction Conditions

- Donor substrate : sucrose (Duchefa Biochemie B.V., Haarlem, Netherlands)
- Acceptor substrate : Daidzin (Extrasynthese, Genay, France)
- Reactor : microtube
- Incubation : Thermal Cycler (Astec Co., Ltd., Fukuoka, Japan)
- Inactivation : 10% DMSO + 90 % Methanol
- Buffer : Tris-HCl, Sodium phosphate

High-performance liquid chromatography (HPLC)

- Column : Cortex 15cm (Waters alliance, MA, USA)
- Detector : PDA15cm (Waters alliance, MA, USA)

Definition of enzyme activity velocity

- Total activity (V_{total}) : Sucrose consumption (= Fructose release)
- 3, 5-dinitrosalicylic acid (DNS)
- *1 unit :* the amount of enzyme that produces 1 μmole of fructose per minute

Effect of pH on the activity

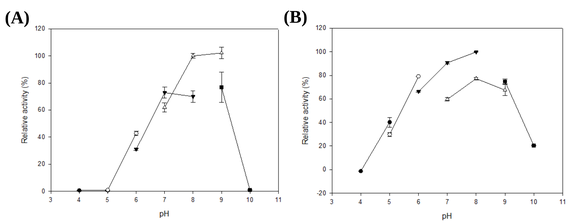


Figure 4. Effect of pH on the activity of NPAS (A) and DGAS (B) ●, sodium acetate pH 4.0 to 5.0; ○, sodium citrate pH 5.0 to 6.0; ▼, sodium phosphate pH 6.0 to 8.0; △, Tris-HCl pH 7.0 to 9.0; ■, and glycine-NaOH pH 9.0 to 10.0. A typical enzyme reaction was carried out at 35 ℃ (NPAS) and 45 ℃ (DGAS) by various pHs

RESULTS

Expression and purification

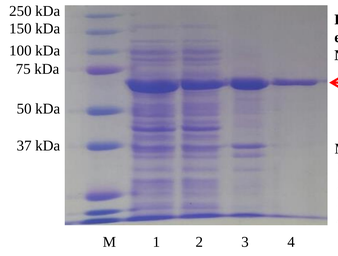


Figure 5. SDS-PAGE analysis of expression and purification of pET-NPAS using affinity chromatography

← Purified recombinant NPAS

M: Molecular standards
1: Crude enzyme
2: Crude passing through enzyme
3. Inclusion body with 8M urea
4. purified NPAS (70 kDa)

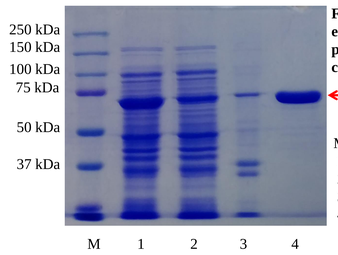
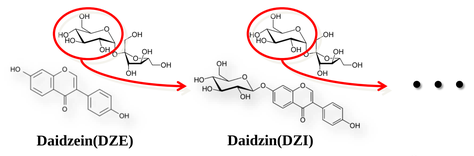


Figure 6. SDS-PAGE analysis of expression and purification of pHCXHD-DGAS using affinity chromatography

← Purified recombinant DGAS

M: Molecular standards
1: Crude enzyme
2: Crude passing through enzyme
3. Inclusion body with 8M urea
4: Purified DGAS (73 kDa)

Transglycosylation aspect



NPAS transglycosylation aspect

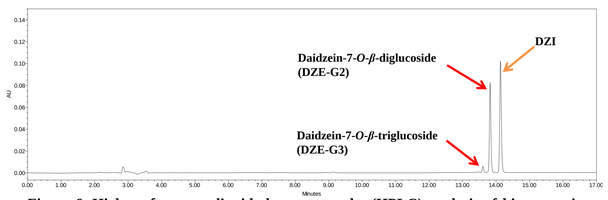


Figure 6. High-performance liquid chromatography (HPLC) analysis of bioconversion reaction by NPAS with daidzin

DGAS transglycosylation aspect

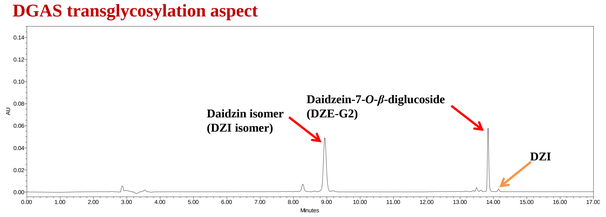


Figure 7. High-performance liquid chromatography (HPLC) analysis of bioconversion reaction by DGAS with daidzin

Buffer & pH dependence

Table 2. The average bioconversion rate of daidzin glycosides by NPAS with different buffers and pHs

	Daidzein-7-glucoside(%)	Daidzin(%)
Tris-HCl pH 7.0	42.7	55.1
Tris-HCl pH 8.0	41.4	56.5
Tris-HCl pH 9.0	31.6	66.4
Sodium phosphate pH 7.0	24.7	72.4
Sodium phosphate pH 8.0	40.5	57.6
Sodium phosphate pH 9.0	41.8	56.4

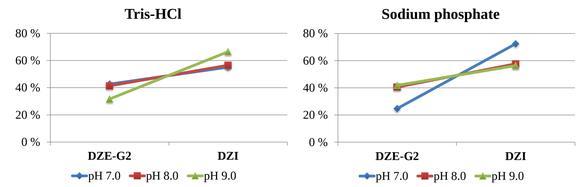
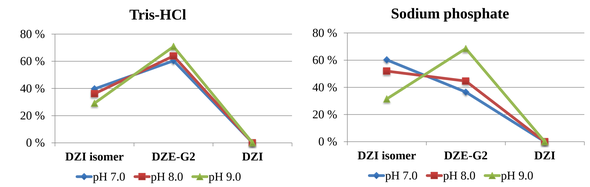


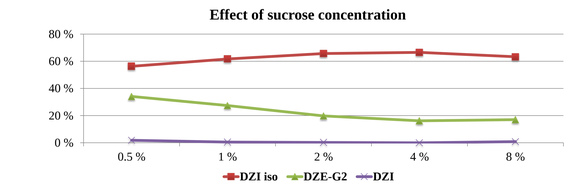
Table 3. The average bioconversion rate of daidzin glycosides by DGAS with different buffers and pHs

	Daidzin isomer(%)	Daidzein-7-diglucoside(%)	Daidzin(%)
Tris-HCl pH 7.0	39.6	60.4	0
Tris-HCl pH 8.0	36.0	63.9	0
Tris-HCl pH 9.0	29.1	70.9	0
Sodium phosphate pH 7.0	60.2	36.5	0
Sodium phosphate pH 8.0	51.9	44.6	0
Sodium phosphate pH 9.0	31.5	68.5	0



Donor substrate concentration dependence

Figure 8. The average bioconversion rate of daidzin glycosides by DGAS with different concentrations of sucrose and daidzin



Effect of reaction time

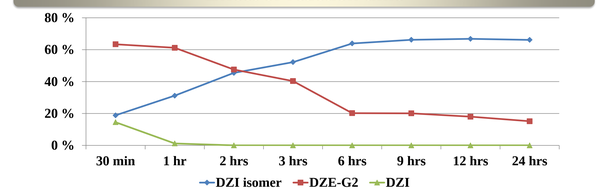


Figure 9. The average bioconversion rate of daidzin glycosides by DGAS with different reaction times

CONCLUSIONS

- The optimum buffer and pH with which NPAS can bioconvert daidzin to daidzein-7-*O*-*β*-diglucoside was Tris-HCl (pH 7.0). But sodium phosphate pH 9.0 also present quite similar activity to Tris-HCl pH 7.0.
- The optimum buffer, sucrose and daidzin concentration that DGAS bioconvert daidzin to daidzin isomer were sodium phosphate pH 7.0, sucrose 4 %.
- In DGAS time dependence reaction, daidzein-7-*O*-*β*-diglucoside is produced higher than daidzin isomer at initial time. But the opposite aspect occurs after 2 hours.

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