KOREAN J. FOOD & NUTR.
Vol. 11, No. 5, 561~564(1998)

Stabilization of Amylolytic Enzymes by Modification with Periodate-Oxidized Soluble Starch

Yong-Geun Ann, Trisaniti Anindyawati*, Kazuo Ito*, Masaru Iizuka* and Noshi Minamiura*

*Dept. of Food and Nutrition, Chungcheong College, Wolkokri, Gangnae, Cheongwon, Chungbuk, 363-890, R. O. Korea, *Dept. of Biology, Faculty of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558, Japan*

과요오드산 산화전분 변형에 의한 아밀라아제의 안정화

안용근 · Trisaniti Anindyawati* · Kazuo Ito* · Masaru Iizuka* · Noshi Minamiura*

충청대학 식품영양과, *大阪市立大學 理學部 生物學科

Abstract

The stabilization of amylolytic enzyme such as β-amylase of barley, β-amylase of wheat, β-amylase of sweet potato, α-amylase of *Bacillus licheniformis*, α-amylase of *Aspergillus* sp. and α-glucosidase of *Aspergillus awamori* was attained by modification with periodate-oxidized soluble starch. The pH stability of modified enzyme was increased at pH 9 for β-amylase of sweet potato, pH 3~5 and 8~11 for β-amylase of barley, pH 2~3 and 7~12 for β-amylase of wheat and pH 6 for α-glucosidase of *Aspergillus awamori*. Thermal stability increased 17.6% for α-amylase of *Aspergillus* sp. at 60℃ for 10min, 30% for α-amylase of *Bacillus licheniformis* at 100℃ for 5min and 4.5% for α-amylase of sweet potato at 60℃ for 10min compared with those of native enzymes.

Key words : stabilization of amylolytic enzymes, modification of amylolytic enzymes, periodate-oxidized soluble starch.

INTRODUCTION

Sweet potato β-amylase (1,4-α-gulcan maltohydrolase, EC 3.2.1.2) is a tetrameric enzyme with a molecular weight of 222,828[1], and consists of four identical polypeptide chains, though most β-amylase are monomeric enzymes. Also, the enzyme loses activity when it dissociates into the monomers at high dilutions[2]. We have reported that the active monomer of sweet potato β-amylase was successfully isolated by modification with periodate-oxidized soluble starch or maltohexaose, and that the tetrameric structure contributed not to catalytic function but to stabilization of the enzyme[3~5]. The stabilization of the enzyme was attained by modification with periodate-oxidized carbohydrates. Industrial enzymes need to have high stability against temperature and pH, but conventional and economically effective methods to give stability to enzymes are very few. The method of modification for enzymes using periodate-oxidized soluble starch is considered to be a simple, economical and convenient method. We attempted to apply this method to industrial amyloytic

Corresponding author : Yong-Geun Ann

enzymes. This paper deals with the method and results obtained.

MATERIALS AND METHODS

1. Preparation of oxidized soluble starch

Sodium periodate was added to the 2% suspension of soluble starch to give final concentration of 0.2M $NaIO_4$ and left to stand with stirring for 24hrs in a cold room (4℃). The oxidized soluble starch was dialyzed against water, then lyophilized.

2. Enzymes

Enzymes used are presented in Table 1. The purity of enzymes employed were electrophoretically homeogenous for sweet potato β-amylase and α-gulcosidase from *Asp. awamori*, and one peak on HPLC with SynChropak GPC 100 column (1×30cm) running in 0.1M K-phosphate buffer containing 0.2M NaCl for wheat β-amylase and α-amylase from *Asp. oryzae* and *Asp.* sp. (Table 1)

3. Activity assay

Enzyme activity was assayed by incubating a mixture of 0.2ml of enzyme and 0.2ml of 2% soluble starch dissolved in 0.05M acetate buffer (pH 5.5), at 37℃ for 10min and determined the reducing sugar formed by Somogyi-Nelson's method[7]. Enzyme activity was defined as the amount of enzyme which produced 1μmole of reducing sugar as maltose or glucose under the above conditions, except in the case of α-glucosidase which was assayed using maltotriitol as a substrate (final conc of substrate, 25%).

4. Modification

A 300units of each enzyme (β-amylase of barley and wheat, α-amylase of *Asp. oryzae*, *Bacillus licheniformis* and *Asp.* sp.) and 2.4mg of oxidized soluble starch dissolved in 0.4ml of 0.25M Tris-HCl (pH 8.0) buffer was left to stand at 37℃ or 40℃ for 10min or 15min to modify enzymes. To test the pH-stability, 50μl aliquots of the reaction mixture was added to 0.95ml of 0.1M Britton-Robinson buffer (of various pHs) and incubated at 37℃ for 2hrs. The mixture was added to 0.1M acetate buffer (pH 5.5) to make a 100-fold dilution. The diluted solution was subjected to an enzyme activity assay. In the case of α-glucosidase from *Asp. awamori*, the enzyme (21units) was reacted with 15ml of oxidized soluble starch in 0.3ml of 0.15M Britton-Robinson buffer (pH 6.0), at 40℃ for 15min. The mixture was then added to 5μl of 50% $NaBH_4$, and incubated at 40℃ for 10min. for the reduction of modified enzyme. The resulting enzyme was added with water (1.22ml) to make a 5-fold dilution. A 120μl aliquots of the mixture was added to 80μl of 0.5M Britton-Robinson buffer, and incubated at 37℃ for 3 hrs. The mixture was then added to a 0.1M acetate buffer (pH 5.5) to make a 10-fold dilution. A 200μl of the diluted solution in a test tube with cap was heated at 100℃, The remaining activity was then measured at 5 minute intervals after diluting with 0.1M acetate buffer (pH 5.5). The native

Table 1. Amylolytic enzymes employed for modification with $NaIO_4$

Enzyme	Activity (u/ml)	Origin	Specification
β-amylase	4,749	Sweet potato	Purified from mashed sweet potato[4]
	10,000	Wheat	Himaltosin GL (Hankyu Bioindustry)
	6,810	Barley	Biozyme ML (Hankyu Bioindustry)
α-glucosidase	2,471	*Asp. awamori*	Purified from culture[6]
α-amylase	135,140	*Asp. oryzae*	Fungamyl (Novonordic Ind. Co.)
	12,766	*Bacillus licheniformis*	Teramyl (Novonordic Ind. Co.)
	7,500	*Asp.* sp.	Bokhabhyoso (Taepyongyang Co.)

unmodified enzyme was treated in the same method in the absence of oxidized soluble starch as in the control. In the case of β-amylase of sweet potato, the enzyme (30units) was reacted with 12.5mg of oxidized soluble starch in 0.25ml of 0.15M Brittion-Robinson buffer (pH 5.0) at 40℃ for 10min. The 10mg of $NaBH_4$ was then added to the mixture and incubated at 40℃ for 15min. The mixture was added to a 0.2M phosphate buffer (pH 6.8) to make 10-fold dilution. A 200μl of the diluted solution was left to stand at various temperatures for 30min, the remaining activity was then assayed.

RESULTS

1. Modification

In HPLC analysis using SynChropak GPC 100 column (1×30cm), retention time of modified enzyme was shifted to a higher molecule than the original enzyme. However the activity of the modified enzyme (β-amylase of sweet potato) was decreased to 91.3% of the original enzyme. All enzymes were modified under a pH range from 2 to 12. The activity of α-glucosidase from *Asp. awamori* treated (control) was reduced to 5% of the original activity, but the activity of the modified enzyme, in the presence of oxidized soluble starch still had a 100%, 95% and 58% for the modification at pH 8, pH 9 and pH 10, respectively. In the results, the highest activities obtained after treatment were pH 8 for α-amylase of *Asp.* sp., pH 8 for α-amylase of *Bacillus licheniformis*, pH 3.5 for β-amylase of sweet potato, pH 8 for β-amylase of wheat and pH 6~9 for α-glucosidase of *Asp. awamori*.

2. pH stability

The pH-stability of α-glucosidase from *Asp. awamori* increased when it modified at pH 6. The stability of other enzymes also increased in stability at pH 3 and between 7 and 12 for β-amylase from barley, and its optimal pH was changed from pH 5 to 7 by the modification. The pH stability of the enzyme increased at pH 9 forβ-amylase of sweet potato, pH 3~5 and 8~11 for β-amylase of barley and 2~3 and 7~12 for β-amylase of wheat. However, the stability of α-amylase of *Asp.* sp. and α-amylase of *Bacillus licheniformis* did not changed. (Fig. 1)

3. Thermal stability

The thermal stability was increased for α-amylase of *Asp.* sp. and *Bacillus licheniformis* as well as β-amylase of sweet potato compared to the stabilities of the original enzymes. Especially, α-amylase of *Asp.* sp. was still stable after 30min at 60℃ as shown in Fig 1. It is reported that the reduction of enzyme with $NaBH_4$ after modification with IO_4-oxidized

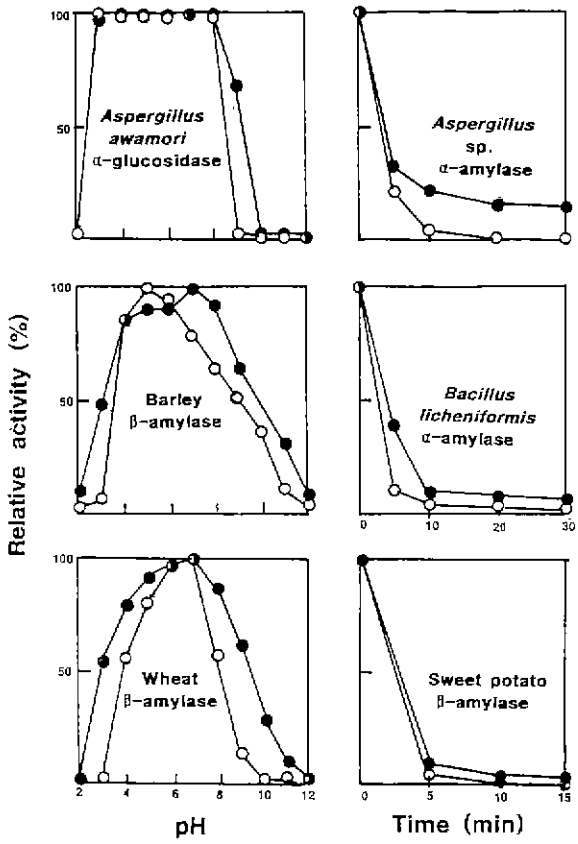

Fig. 1. pH- and thermal-stabilities of amylolytic enzymes modified with IO_4-oxidized soluble starch. Conditions, see the text. ○, native enzyme ; ●, modified enzyme.

soluble starch. It was observed that stability increased for β-amylase from barley and wheat when it was subjected to the treatment both with and without reduction, whereas α-glucosidase from *Asp. awamori* increased only when it was subjected to reduction with $NaBH_4$. It was observed that the thermal stability of α-amylase of *Asp.* sp. and *Bacillus licheniformis* increased both cases of both treatment, with and without reduction. However stability of sweet potato β-amylase increased when the modified enzyme was reduced with $NaBH_4$. (Fig. 1)

DISCUSSION

The stabilization of enzyme by modification with IO_4-oxidized carbohydrate is considered due to the introduction of carbohydrate to the surface of enzyme, by formation of Schiff base, as reported previously[4]. There are several methods that enable the producer of a more stable enzyme such as immobilization, chemical modification, and screening of the enzyme producer. In this paper, we have suggested that IO_4-oxidized soluble starch can be used for modification of many enzymes. The modification procedure is simple and easy to complete in a short time at low temperatures (25 to 40℃). Furthermore soluble starch, a material for modification, is considered one of the cheapest raw materials available. As well, the method may be used to modify some physicochemical properties such as, digestability, stability, solubility, viscosity, etc., to obtain a desired properties of protein in the food, the medicine and the cosmetic industry.

REFERENCES

1. Toda, H., Nitta, Y., Asanami S., Kim, J.P. and Sakiyama, F. : Sweet potato β-amylase ; primary structure and identification of the active-site glutamyl residue, *Eur. J. Biochem.*, 216, 25-38 (1993).
2. Bernfeld, P., Berkeley B.J. and Beiber R.E. : Reversible dissociation of enzymes of high dilutions and their inhibition by polyanions, *Arc. Biochem. Biophys.*, 111, 31-38 (1965).
3. Ann, Y.G., Iizuka, M., Yamamoto, T. and Minamiura, N. : Evidence of an active monomer of sweet potato β-amylase, *Agric. Biol. Chem*, 53, 3109-3110 (1989).
4. Ann, Y.G., Iizuka, M., Yamamoto, T. and Minamiura, N. : Preparation and some properties of active monomer of sweet potato β-amylase, *Agric. Biol. Chem.*, 54, 769-774 (1990).
5. Ann, Y.G., Iizuka, M., Yamamoto, T. and Minamiura, N. : Active monomer of sweet potato β-amylase ; Stabilization and an improved preparation method using α-cyclodextrin, *J. Ferm. Bioeng.*, 54, 75-19 (1990).
6. Anindyawati, T., Ann, Y.G., Ito, K., Iizuka, M. and Minamiura, N. : Two kinds of novel -glucosidase from *Aspergillus awamori* KT-11 ; Their purification, properties and specificities, *Biosci. Biotech. Biochem.*, 85, 465-469 (1995).
7. Nelson, N. : A photometric adaption of the Somogyi method for determination of glucose, *J. Biol. Chem.*, 153, 375-379 (1944).
8. Kobayashi, M. and Takatsu, K. : Cross-linked stabilization of trypsin with dextran-dialdehyde, *Biosci. Biotech. Biochem.*, 58, 275-278 (1994).

(Received December 15, 1998)