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Comparison of Soybean and Sweet Potato β-Amylases

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대두 및 고구마 β-Amylase의 비교에 관한 연구

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초 록

대두 및 고구마로부터 얻은 β-Amylase의 단백질 구조를 CD Spectra, 항체반응, 화학적 절단을 통하여 비교하였다. 고구마 β-Amylase는 4개의 동일한 subunit로 구성되어 있으며 대두 β-Amylase는 Subunit구조를 하고 있지 않았다. 또한 두 효소는 변성시킨 상태에서 SDS-gel전기영동, gel filtration한 결과 분자량은 동일하였다. 그리고 대두 및 고구마 β-Amylase는 CD spectra상 유사한 2차구조를 나타내고 있으나 방향족 측쇄가 상이함을 나타냈다. 한편 cyteine 잔기 및 methionine 잔기의 화학적 절단한 결과 두 효소는 동일한 아미노산 배열을 나타냈다. 또한 면역학적인 방법에 의해서도 두 효소는 유사성이 인정되었다. 한편 대두 β-Amylase에 대한 항체는 고구마 β-Amylase의 활성을 억제하였으나 밀, 보리, 무우 β-Amylase에 대해서는 활성 억제가 나타나지 않았다.

Introduction

β-Amylase [α-1, 4-glucan maltohydrolase, EC. 3.2.1.2] catalyzes the liberation of β-anomeric maltose from non-reducing ends of α-1, 4-glucan such as starch and glycogen. The enzyme is distributed in higher plants and some microorganisms. The enzyme purified from soybean[1,2], wheat[3], Japanese radish[4,5], barley[6], rice[7] and malted sorghum[8] are monomeric enzymes having molecular weight of 50,000~60,000 daltons, whereas only sweet potato β-amylase [9~11] is a tetrameric enzyme having four identical subunits of 50,000 daltons. These plant-type β-amylase has similar properties, in terms of amino acid composition, isoelectric point, optimum pH and inactivation by SH-blocking reagents[12,13]. In spite of the importance of β-amylase in food industries and the accumulated knowledge about kinetic properties of this enzyme, the primary and the three-dimentional structure of the enzyme has not been given. Though the technological importance of β-amylase from soybean and sweet potato is evident as the enzyme source for industrial application, the difference of their enzymatic properties has not been clarified.

In this report, we describe the comparison of enzymatic properties between soybean and sweet potato β-amylases. The similarity was demonstrated on immunological reaction, circular dichroism and peptide-mapping.

Materials and Methods

β-amylase

Soybean β-amylase (isozyme 2) was purified from defatted soybean whey by the method of Mikami et al.[12]. Sweet potato β-amylase was purified according to Kim et al.[13]. β-Amylases from Japanese radish and wheat were purified by the method of Morita and Yadano[4] and Kato et al.[14], respectively. Barley β-amylase was purchased from Fluka Co., Switzerland. The exticntion coefficients ($E^{1\%}$ 280nm) used were 17.0 and 16.5 for soybean[2] and sweet potato[15] β-amylases, respectively. The activity of β-amylase was determined by the method of Bernfeld[16] using amylopectin as the substrate in 0.1M acetate buffer, pH 5.4 at 30°C for 3 min.

Measurement of circular dichroism

Circular dichroism(CD) was measured at room temperature using a JASCO J-500C spectropolar imeter. Cells having a light path of 0.1mm and 10mm were used for the measurements in far and near ultraviolet wavelength region, respectively.

Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli[17] using 7% and 15% polyacrylamide gel, for estimation of molecular weight and separation of fragments produced by chemical cleavage, respectively.

Preparation of antibody against soybean β-amylase isozyme 2

Antisera against purified soybean β-amylase were obtained by the immunization of young adult rabbits at two weekly intervals with 3 injections each containing 8~10mg protein. The injections were given with complete Freund's adjuvant. The γ-globulin fraction was obtained by repeated precipitation with ammonium sulfate at 33% saturation.

Chemical cleavage method

Specific cleavage of soybean and sweet potato β-amylases at cystein residues was carried out by the method of Jacobson et al.[18] using 2-nitro-5-thiocyanobenzoic acid (NTCB) as a cyanylation reagent. Specific cleavage of the enzymes at methionine residues was performed acco rding to Gross & Witkop[19]. The chemical cleavage by 0.015N HCl and by 80% formic acid was carried out according to Marcus[20] and Landon[21], respectively.

Results and Discussion

Immunological reactivity of β-amylases

Figure 1 shows the reaction of β-amylases with the antibody against soybean β-amylase isozyme 2 followed by the enzymatic activity. Except for the rapid decrease of the enzymatic activity of soybean enzyme, the marked inactivation of sweet potato enzyme was observed. But the inactivation of barley, wheat and Japanese-radish β-amylase was not observed. The inactivation of soybean and sweet potato β-amy lase suggests that these enzymes have the same structure at least at the site of antibody formation.

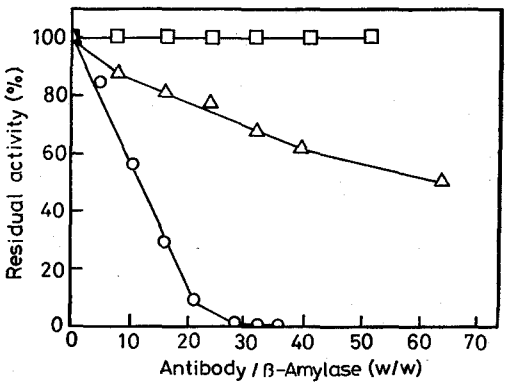

Fig. 1. Inactivation of β-amylases from various higher plants by the antibody against soybean β-amylase. β-Amylase from various higher plants(0.7—0.4μM) were incubated with the indicated concentration of γ-globulin against soybean β-amylase at 0°C for 1h and the residual activity was measured. □, represents β-amylase from barley, wheat and Japanese radish; △ and ○, β-amylase from sweet potato and soybean, respectively.

CD spectra of soybean and sweet potato β-amylase

Figure 2 shows the CD spectra of soybean and sweet potato β-amylase. In the far ultraviolet, the spectra are almost same shape, exhibiting the two negative bands around 210 and 222nm and a positive band in the 190~195nm. The spectral shapes correspond to that of a typical α/β-protein according to the classification of Manavalan and Johnson[22]. The content of α-helix and β-sheet structures were estimated by the method of Henessy and Johnson[23] to be 22 % and 18% for soybean β-amylase, and 18% and 21% for sweet potato β-amylase, respectively. In contrast to the similarity in the far ultraviolet region, the spectra in the near ultraviolet region considerably different between the two enzymes. The peaks at 292 and 297nm, at 273 and 281nm, and at 259 and 265nm of soybean enzyme were suggested to be assigned to tryptophanyl, tyrosyl and phenylalanyl chromophores, respectively[24]. The spectrum of sweet potato enzyme shows the peaks at the corresponding wavelength to the soybean enzyme but the height of the peaks were different, suggesting that the number and circumstance of the aromatic amino acids of sweet potato enzyme differ from those of soybean enzyme.

Molecular weight

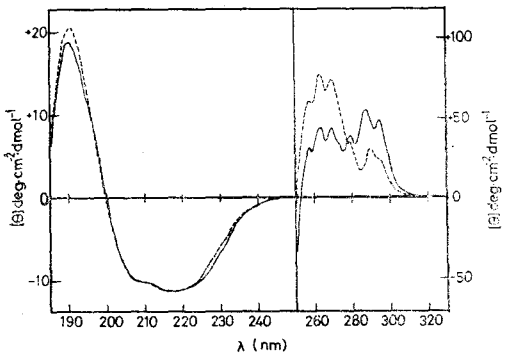

Fig. 2. CD spectra of soybean and sweet potato β-amylase. The spectra were measured in 0.1M acetate buffer, pH 5.4 at a protein concentration of 1.0mg/ml. ——, soybean β-amylase;·····, sweet potato β-amylase.

Prior to the study of chemical cleavage, the monomer molecular weights of soybeen and sweet potato β-amylase were estimated by HPLC method[25] (Fig. 3). The molecular weight of soybean and sweet potato β-amylase were estimated to be 58,000±2,000 and 57,000±2,000, respectively. The values estimated on SDS-polyacrylamide gel were 57,000±2,000 and 56,500 ±2,000, respectively. These results indicate that the monomer molecular weights of the two amylases are almost same except that the sweet potato enzyme forms tetramer in the native state.

Chemical cleavage of β-amylase

In order to compare the structure of soybean and sweet potato β-amylase, these enzymes were cleavaed at the specific amino acid residues by chemical cleavage method. Figure 4 shows the fragments obtained by the cleavage at cysteinyl residues of the enzymes. The number of cyste-

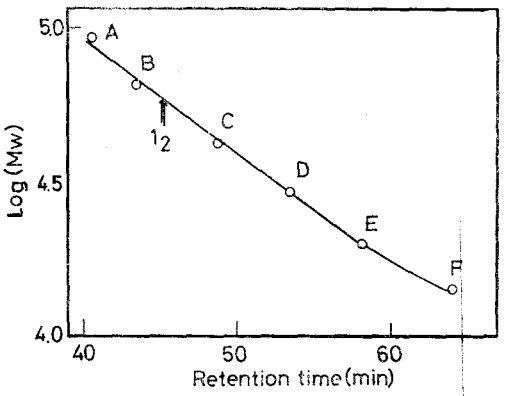

Fig. 3. Estimation of molecular weight of soybean and sweet potato β-amylase on a HPLC column.

The column of G3000 SW (7.5×600 mm) was equilibrated with 0.2M Na/phosphate buffer pH 6.8, containing 0.2% SDS at a flow rate of 0.3ml/min. The molecular weight markers used were A, phosphorylase b (Mw 94,000); B, bovine serum albumin(Mw 67,000); C, eggalbumin (Mw 45,000); D, carbonic anhydrase (Mw 30,000); E, soybean trypsin inhibitor(Mw 20,100); and F, β-lactalbumin (Mw 14,400). 1, soy bean β-amylase; 2, sweet potato β-amylase.

ine residues is reported to be 6 and 5~7 for soybean[26] and sweet potato[11,25] enzymes, respectively. Comparison of the fragments of sweet potato β-amylase with that of soybean enzyme reveals that the fragments of 47,000, and 35,000 daltons are common but the molecular weight of other fragments differs considerably, suggesting the different distribution of cysteine residues along the polypeptide chains. The fragments of 47,000 and 35,000 daltons of soybean enzyme are known to produce by cleavage at SH2 and SH5[27]. The results revealed the conservation of these SH groups between soybean and sweet

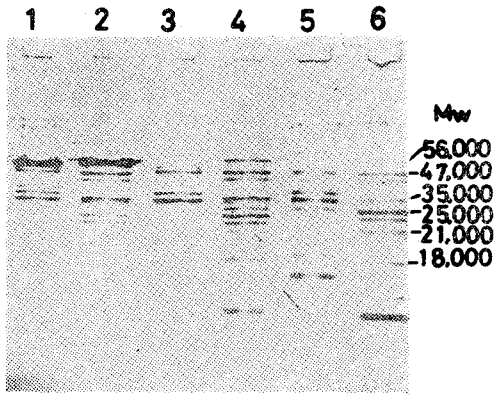

Fig. 4. Specific cleavage of soybean (Lanes 2, 4 and 6) and sweet potato (lanes 1, 3 and 5) β-amylase at cysteine residues.

The enzymes (1mg each) was incubated with 2mM 2-mercaptoethanol in 0.1M Tris-HCl buffer, pH 8, containing 6M guanidine-HCl and 5mM EDTA for 30 min at 30°C. The denatured enzymes were treated with 7mM NTCB for 1h at 30° for cyanilatation of cysteine residues. Then, the cleavage reaction was performed by increasing the pH of the reaction mixture to 9 and temperature to 37°C. After the aliquots of the mixture was treated with 50mM 2-mercaptoethanol to stop the cleavage reaction, it was analyzed on SDS-gel electrophoresis after guanidine-HCl was removed on a Sephadex G-25 column equilibrated with 0.05M Tris-HCl buffer pH 7, containing 6M urea and 20mM 2-mercaptoethanol. Lanes 1 and 2, 0 time cleavage; lanes3 and 4, 1h cleavage; lanes 5 and 6, 24h cleavage.

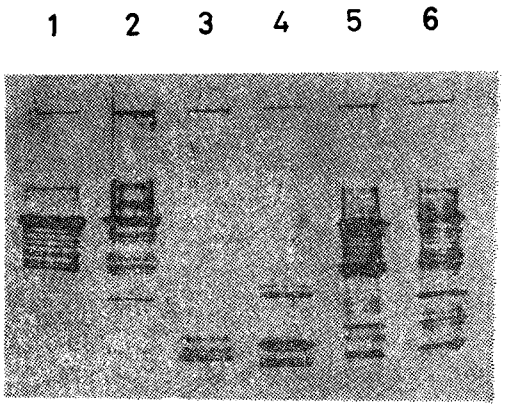

Fig. 5. Chemical cleavage of soybean and sweet potato β-amylase by 80% formic acid, cyanogen bromide and 0.015N HCl. The enzymes (1mg each) were cleaved by 80% formic acid at 30°C for 24h(lanes 1 and 2), by cyanogen bromide in 70% formic acid for 24h at 25°C(lanes 3and 4) and by 0.015N HCl for 5 min at 100°C (lane 5 and 6) and the produced fragments were analyzed on SDS-gel electrophoresis after dilution with water and lyophilization. Lanes 1, 3 and 5, sweet potato β-amylase; lanes 2, 4 and 6, soybean β-amylase.

potato enzyme. SH2 of soybean β-amylase is the second reactive residue and it is responsible for the inactivation of the enzyme by SH-blocking reagents. Thoma et al. reported that the chemical modification of SH groups in sweet potato enzyme also leads to the inactivation but that SH groups do not participate the catalytic action of the enzyme[28]. Recent study of SH2 of soybean enzyme[29,30] demonstrated that the modification of SH2 inhibited the binding of glucose to the enzyme depending on the bulkiness of the substituent at the SH group, suggesting that the SH2 is located at or near the substrate binding site (subsite 1). Thus the amino acid sequence and the conformation around SH2 of soybean β-amylases seems to be conserved in sweet potato enzyme.

Figure 5 shows the cleavage of soybean and sweet potato β-amylase by 80% formic acid, cyanogen bromide and 0.015N HCl. In the cleavage by formic acid and HCl, sweet potato

enzyme produced large molecular weight fragment of 54,000 (Fig. 5 lane 1 and 5) indicating that specific cleavage occurred near N- or C-terminus of the enzyme, but it was not observed for the cleavage of soybean enzyme. The cleavage site of sweet potato enzyme may be assigned to Asp-Pro bond[19,20]. In the cleavage using cyanogen bromide (Fig. 5 lane 3 and 4), the fragments patterns of the two enzymes are need to be reworded except for somewhat large molecular weight fragment (Mw. 18,000) of soybean enzyme. The similarity suggests the similar distribution of considerable methionine residues on the polypeptide chains of the enzymes. As the summary of the cleavage study, it is suggested that the amino acid sequence of soybean and sweet potato β-amylase are different in the region which is not required for the maintenance of the active enzyme conformation.

Acknowledgement

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Abstract

The enzymatic properties of β-amylase from soybean and sweet potato were compared. The sweet potato enzyme consists of four identical subsunits whereas soybean enzyme has no subunit structure[12,15]. In the denatured state, both enzymes exhibited the same molecular weight on SDS-gel electrophoresis and on gel-filtration analysis. The spectra of circular dichroism revealed that both enzyme have almost same secondary structure but the environment of aromatic side chains are different. The chemical cleavage of soybean and sweet potato β-amylases at cysteine residues and methionine residues demonstrated the homology of amino acid sequence between the enzymes. The similarity between soybean and sweet potato β-amylase was also revealed by immunological method. The antibody for soybean enzyme inhibited the activity of sweet potato enzyme but it did not inhibit the activity of wheat, barley and Japanese-raddish β-amylases.

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