Purification and Biochemical Analysis of Rice Bran Lipase Enzyme

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쌀겨로부터 lipase 효소의 정제 및 생화학적인 분석

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1. Abstract

A simple procedure for the extraction of the lipolytic enzyme from rice bran has been developed. High activity of lipolytic enzyme was obtained by first defatting the rice bran to remove lipid components with various extraction conditions. Then, after five cycles of aqueous extraction, rice bran lipolytic enzyme was purified using micro- and ultrafiltration apparatus. Lipolytic enzyme activity was estimated by its hydrolytic action of tributyrin. The result indicated that the standard activity curve of butyric acid showed that the potential rice bran enzyme is a hydrolytic lipase enzyme. In addition, it showed higher lipolytic activity and specific enzyme activity with further purification by micro- and ultrafiltration.

1. Introduction

Lipases are enzymes that are primarily responsible for the hydrolysis of acylglycerides. However, a number of other low- and high-molecular weight esters, thiol esters, amides, polyol/polyacid esters, etc. are accepted as substrates by this unique group of enzymes. A variety of reactions are further enlarged by the fact that lipases are capable of catalyzing the reverse reaction of synthesis just as efficiently. In

this research, the biochemical activity of rice bran lipase was observed by using the aqueous extract after membrane filtration.

2. Materials and Methods

2.1. Defatting of rice bran

1 kg of rice bran(*Oryza sativa* cv. Chuchung) was purchased from a local mill (Mokcheon, Cheonan). 10 g of milled rice bran was stirred with 30 mL of n-hexane for 30 min for defatting. At the end of stirring period, the hexane phase was decanted and 30 mL of fresh n-hexane was added to the rice bran. This procedure was repeated twice.

2. 2. Extraction of rice bran lipase

In order to remove the hexane completely, the defatted rice bran from above was dried in air about 1 hour and then used for extraction. For this purpose potassium phosphate buffer containing calcium chloride was used. The 50 mM phosphate buffer containing 0.5 mM calcium chloride was used to get a maximum activity in the extract.

2. 3. Lipase assay

The standard activity curve of butyric acid was required to measure the decomposed amount of tributyrin because lipase is an enzyme to catalyze the hydrolysis of tributyrin. Lipase activity was estimated by its hydrolytic action of tributyrin, which was emulsified in various emulsifying agents such as gum acacia. A 2 mL portion of 7.5% gum acacia was mixed with 10 mL of enzyme extract. To this 1 mL of tributyrin was added, and the mixture was stirred for 1 min with a magnetic needle on an orbital shaker at 30℃.

3. 3. Calculation of lipase activity

Lipolytic activity is expressed in terms of lipase units (U) per unit weight of rice bran (in kg). One unit is defined as the micromoles of butyric acid liberated by the hydrolysis of tributyrin at 3 0℃ per minute. The amount of acid released can be calculated from the difference in titer values of a sodium hydroxide solution of known molarity. Specific activity was calculated as the units per mg protein.

3. Results and Discussion

3.1. Effect of substrate

In order to test the biochemical activity of rice bran lipase, the hydrolytic action of tributyrin was estimated for the its reaction rate. Lipolytic activity is expressed in terms of lipase units (U) per unit weight of rice bran (in Kg). One unit is defined as the micromoles of butyric acid liberated by the hydrolysis of tributyrin. The amount of acid released was calculated from the difference in titer values of a sodium hydroxide solution of known molarity.

It was found that the amount of sodium hydroxide used was proportional to that of liberated tributyric acid. It was clear that the rate of hydrolysis was highest in the case of tributyrin. Among the oils, it showed that olive oil was hydrolyzed faster than the other oils. Other study on pancreatic lipase have yielded similar results, namely, that shorter chains are hydrolyzed by the

lipase enzyme faster than longer chains. In addition, oleyl chains have been reported to be released slightly faster than other long chain-fatty acids (Aizono et al. 1973).

3. 2. Aqueous extraction and membrane filtration of the rice bran lipase

The protein distribution obtained during this purification process is shown in Table 3. Enzyme activity in the MF extract was increased from 3.6 × 10^{-7} to 6.0 × 10^{7} U/mL. Generally amount of total protein (mg) was decreased. However, enzyme activity (U/mL) and specific activity (U/mg) were increased. Enzyme activity in the MF extract was increased only marginally from 3.6 × 10^{7} U/mL to 6.0 × 10^{7} U/mL. But specific activity in the MF extract was increased from 4.0 x 10^{9} (U/mg) to 7.3 x 10^{8} U/mg. An 18-fold increase in specific activity of the MF extract was obtained by microfiltration using the PVDF membrane.

4. References

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