

Note

STUDY ON HYDROLYSIS OF MACROMOLECULAR GELATIN WITH ENZYMES IN COMBINATION MODE*

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Abstract The enzymatic hydrolysis of macromolecular gelatin with AS1.398 neutral protease, bromelain and their combinations was studied by estimating the molecular weights of their hydrolytic products. It was discovered that the products hydrolyzed by using combination enzymes had lower molecular weight than those obtained by using single ones, and the kind of enzymes, their combination mode and addition sequence are effective ways to control the molecular weights of gelatin hydrolyzates.

Keywords Gelatin hydrolyzate, Enzyme, Gelatin

INTRODUCTION

Macromolecular gelatin is principally derived from type I collagen. Gelatin hydrolyzate can be prepared from macromolecular gelatin by hydrolytic reaction with acid, alkali, heating or enzyme.

The gelatin hydrolyzate maintains most of the inherent physical and chemical characteristics of macromolecular gelatin and also acquires some new ones by taking advantage of the lowering of the molecular weight. With the advancement of science and technology, the application of gelatin hydrolyzate becomes broader, especially after its curative effect has been discovered. Therefore, consumers demand gelatin hydrolyzate of better and better quality. The enzymatic method is the most appropriate one, for preparing gelatin hydrolyzate in a controlled hydrolytic process, and good quality of products can be easily reached. Nowadays, gelatin hydrolyzate prepared by a single enzyme has been studied by many researchers^[1–8], but that produced with combination enzymes has scarcely been studied.

An enzyme is a kind of biological catalyst with high catalytic efficiency and specificity. Different enzymes have different specificities. So, preparation of gelatin hydrolyzate by using combination enzymes consisting of two or more enzymes showed yield products with even lower molecular weights and narrower distribution and it should also be easier to study the relationship between their function and the structure. In this paper, the preparation of gelatin hydrolyzate by using combination enzymes consisting of AS1.398 neutral protease and bromelain combined in different modes was studied.

EXPERIMENTAL

Materials

Macromolecular gelatin (medical grade) was obtained from Rousselot (Great China) Gelatin Co. LTD; Bromelain was supplied by Beijing Huaxin Co., and has an activity of 500–600 tyrosine units (U)/g; AS1.398

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protease was obtained from Wuxi Enzyme Agent Co., and has an activity of 100 tyrosine units (U)/mg; all other reagents were of analytical grade.

Methods

Hydrolysis by single enzyme

A quantitative amount of macromolecular gelatin solution (its pH value was adjusted with 0.5 mol/L citric acid solution or 0.2 mol/L dibasic sodium phosphate solution) was placed in a water-bath controlled at a certain temperature. Then, a quantitative amount of single enzyme solution was added to the macromolecular gelatin solution and stirred gently and continuously to start the reaction. After the hydrolytic reaction had proceeded to a certain degree, the enzyme was deactivated at high temperature, and the hydrolytic solution was filtered. The filtrate was dehydrated and was ready for testing.

Hydrolysis by combination enzymes

A quantitative amount of macromolecular gelatin solution (its pH value was adjusted with 0.5 mol/L citric acid solution or 0.2 mol/L dibasic sodium phosphate solution) was placed in a water-bath controlled at a certain temperature. The first kind of enzyme solution was added to the macromolecular gelatin solution, and the solution stirred gently and continuously. After the hydrolytic reaction had proceeded to a certain degree, the enzyme was deactivated at high temperature, the solution filtered, and the pH of the obtained primary product solution was adjusted to a certain value, and was again placed in a water-bath controlled at a certain temperature. Then, another kind of enzyme solution was added to the above solution to continue the hydrolytic reaction. After a given time the enzyme was deactivated at high temperature, and the obtained solution was filtered. The filtrate was dehydrated and the final hydrolytic product was ready for test.

Measurements

Estimation of molecular weight

4% gelatin hydrolyzate solution prepared with 0.5 mol/L sodium chloride solution was heated and then cooled down rapidly to the room temperature. 0.25 mL solution sample was injected into a column filled with Dextran G-50 (Pharmacia) and eluted with 0.25 mol/L NaCl solution at a flow rate of 9–10 mL/h to carry out the chromatographic separation. The absorbance of the eluent fractions (1.5–1.6 mL/fraction) was determined by an UV spectrophotometer at 230 nm. Molecular weight of the samples could be calculated according to the data obtained.

The molecular weight was estimated by using gel filter chromatography^[9].

RESULTS AND DISCUSSION

Hydrolysis by Single Enzyme

The experimental results of gel filtration chromatography are shown in Fig. 1.

The kind of enzyme used has a great effect on the elution curves of the hydrolytic products. Curve a has the eluting peak appearing earlier and broader, and the eluting peak of curve b appears later and narrower. This indicates that the average molecular weight of the sample hydrolyzed by AS1.398 neutral protease is relatively higher, and its molecular weight distribution is broader; while the average molecular weight of the sample hydrolyzed by bromelain is comparatively lower, and its molecular weight distribution is narrower. Their calculated values are as follows: $M_n = 4390$, $d = 1.25$ for the sample hydrolyzed by AS1.398 neutral protease, and $M_n = 3350$, $d = 1.30$ for the sample hydrolyzed by bromelain. It is obvious that the difference in the molecular parameters of the two hydrolytic products is mainly due to the difference in specificity of the two enzymes.

Hydrolysis by Combination Enzymes

The results obtained from the gel filtration experiments for combination enzymes are shown in Fig. 2, and the calculated data are summarized in Table 1.

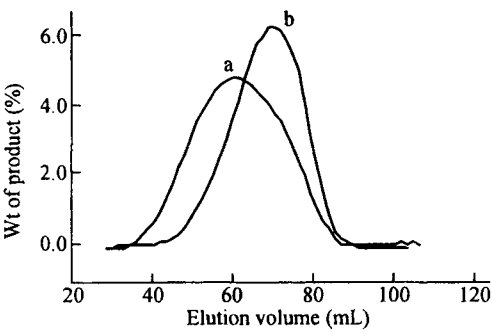


Fig. 1 Elution curve of gelatin hydrolyzates obtained by using a single enzyme
a) Gelatin hydrolyzate obtained using AS1.398 neutral protease; reaction time 30 min; reaction temperature 45°C; pH value 7.0; gelatin concentration 5%; enzyme amount 1% (relative to the weight of the dried gelatin)
b) Gelatin hydrolyzate obtained using bromelain; reaction time 2 h; temperature 45°C; pH value 7.0; gelatin concentration 5%; enzyme amount 1% (relative to the weight of the dried gelatin)

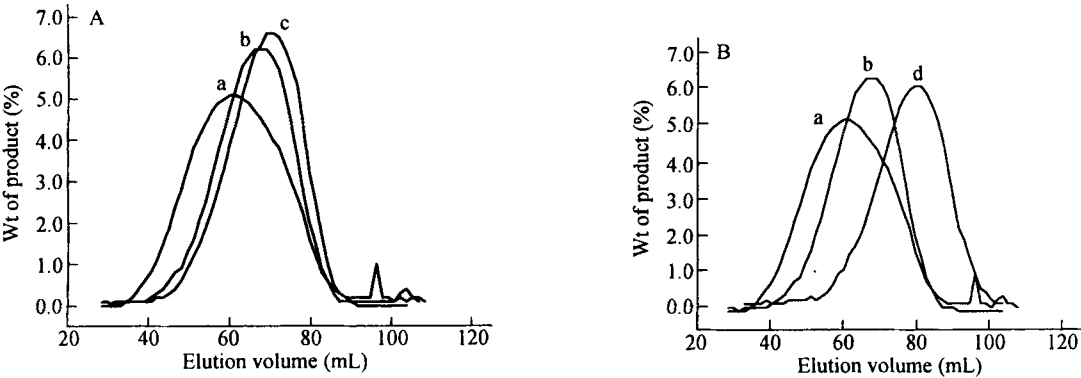


Fig. 2 Elution curve of gelatin hydrolyzates obtained by using combination enzymes
a) Gelatin hydrolyzate obtained using AS1.398 neutral protease
b) Gelatin hydrolyzate obtained using bromelain
c) Gelatin hydrolyzate obtained using combination-enzymes (hydrolyzing by AS1.398 neutral protease first, followed by bromelain)
d) Gelatin hydrolyzate using combination-enzymes (hydrolyzing by bromelain first, followed by AS1.398 neutral protease)

Table 1. Molecular weights and their distributions of gelatin hydrolyzates

Enzyme	Using mode	M_w	M_n	D
AS1.398 neutral protease	Single	5470	4390	1.25
Bromelain	Single	4340	3350	1.30
AS1.398 neutral protease+ Bromelain	Combination	3970	3260	1.22
Bromelain+AS1.398 neutral protease	Combination	2790	2130	1.30

From the above experimental results, it could be concluded that a combination of two different enzymes is more effective for hydrolyzing gelatin than a single enzyme. The product obtained has lower molecular weights, moreover it was found that the addition sequence of the two different enzymes also played an important role in adjusting the molecular weight of the gelatin hydrolyzate in the hydrolytic reaction. The curve c (Fig. 2a) demonstrates the elution curves of hydrolysis sample obtained by using AS1.398 neutral protease first, followed by bromelain. The elution volume of its peak is 70 mL. Similarly, the curve d (Fig. 2b) demonstrates the elution

curves of hydrolysis sample obtained by using bromelain first, followed by AS1.398 neutral protease. The corresponding peak elution volume is 85 mL. It is evident that the hydrolyzed product obtained by using AS1.398 protease first has higher molecular weight than the one by using bromelain first, and the calculated data listed in Table 1 support these results.

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