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Improvement of a method for chain-length distribution analysis of wheat amylopectin

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Abstract

For the chain-length distribution analysis of wheat amylopectin using high-performance anion-exchange chromatography (HPAEC) under alkaline conditions, the reduction of amylopectin isoamylolyzate with sodium borohydride facilitated obtaining reproducible chromatograms without both alkaline degradation and epimerization of maltooligosaccharides in aqueous NaOH solution. In a comparison of the chromatograms of isoamylolyzates of wheat starch and its fractionated amylopectin, reproducible results were also obtained. The improved method presented here, based on the use of starch isoamylolyzates reduced with sodium borohydride, is available for performing automated analysis of the chain-length distribution of wheat amylopectin by HPAEC equipped with an autosampler.

Keywords: Chain length distribution; Amylopectin; Starch; Maltooligosaccharides

1. Introduction

A simple and automated method for the chain-length distribution analysis of wheat amylopectin is necessary for the screening of wheat breeding lines possessing the preferable starch especially for manufacturing Japanese noodles, because the chain-length distribution characteristics are known to influence the physicochemical properties of starch in addition to amylose content [1,2].

High-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection has recently been used for the chain-length distribution analysis of amylopectins of higher plants [3] or of oyster glycogen [4,5]. HPAEC can precisely separate maltooligosaccharides according to their degree of polymerization (DP) up to more than 50 under alkaline conditions [6]. However, under alkaline conditions, the degradation and epimerization of several kinds of oligosaccharides have been reported [6–8]. In order to develop an automated method for the chain-length distribution analysis of amylopectin using an autosampler, which is useful for the analysis of a large number of wheat samples under the same conditions of HPAEC, it is necessary to avoid both alkaline degradation and epimerization of maltooligosaccharides which are released from amylopectin by the treatment with isoamylase.

In this paper, the change in maltooligosaccharides in alkaline solution and the effect of the reduction of maltooligosaccharides with sodium borohydride to avoid both their alkaline degradation and epimerization were investigated using both the standard maltooligosaccharides and the maltooligosaccharides derived from the isoamylosis of the wheat amylopectin. In the course of this study, furthermore, the

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influence of the use of starch isoamylolyzate instead of amylopectin was also investigated to simplify the sample preparation for the chain-length distribution analysis of wheat amylopectin.

2. Experimental

2.1. Materials

Maltotetraose, maltopentaose, maltohexaose and maltoheptaose, which were used as the standard maltooligosaccharides, and isoamylase (EC 3.2.1.68) from *Pseudomanas amyloderamosa* were purchased from Hayashibara Biochemical Lab. (Okayama, Japan).

The starch and amylopectin for the chain-length distribution analysis of amylopectin were isolated from the flour of the wheat cultivar Kanto 107. An amount of 200 g of flour and 80 ml of water were mixed to produce a stiff dough. Starch was then extracted from the dough by hand-washing in ice-cold water, and the residual proteins were removed with toluene using the method of Adkins and Greenwood [9]. The separation of amylopectin from starch was carried out using the method of Takeda and Hizukuri [10].

2.2. Preparation of isoamylolyzate of amylopectin or starch and the reduction of isoamylolyzates and standard maltooligosaccharides

A sample of amylopectin or starch (30 mg) was suspended in distilled water (30 ml) in a capped vessel and then boiled at 100°C for 30 min. After cooling the sample solution to room temperature, the reaction mixture containing a 0.1% solution of amylopectin or starch (930 μl), 500 m*M* acetate buffer (50 μl, pH 4.7), 2% sodium azide (10 μl) and isoamylase (700 units) was incubated with gentle stirring in a capped test tube at 40°C for 24 h. After the incubation, the digested solution was lyophilized with or without reduction with sodium borohydride. The reduction of isoamylolyzate was performed by the addition of 10 mg/ml sodium borohydride (50 μl) after adjustment of the digested solution to pH 9.0 by the addition of 6% ammonium aqueous solution (60 μl). The solution was then allowed to stand for 24 h at room temperature. The reduction of standard maltooligosaccharides, which dissolved with water at a concentration of 0.5 mg/ml, was carried out using the same method as that for isoamylolyzate. The reduced products were also lyophilized and then used for the HPAEC analysis.

2.3. Analysis of maltooligosaccharides by high-performance anion-exchange chromatography (HPAEC)

In order to prepare the sample solution of standard maltooligosaccharides for HPAEC, standard maltooligosaccharides were dissolved with 100 m*M* NaOH at a concentration of 25 μg/ml. The non-reduced isoamylolyzates were, in principle, dissolved first with 400 m*M* NaOH (500 μl) and then diluted to prepare the sample solution in 100 m*M* NaOH with distilled water (1500 μl) at 30 min after the addition of 400 m*M* NaOH. Some isoamylolyzates were directly dissolved in 100 m*M* NaOH (2000 μl) to investigate the influence of NaOH concentration on the solubility of the isoamylolyzates. With the dissolution of the reduced isoamylolyzate, 1 *M* NaOH (200 μl) and distilled water (1800 μl) were used. The dissolved digest was centrifuged (2000 *g*, 5 min) and then passed through a 0.5-μm PTFE membrane filter (Advantec, Tokyo, Japan) followed by HPAEC analysis.

The HPAEC was conducted with the DX-300 system (Dionex, CA, USA) equipped with a pulsed amperometric detector (Model PED). The column used was a CarboPac PA-1 (250×4 mm I.D.) with a CarboPac PA-1 guard column (25×3 mm I.D.). For the HPAEC analysis, 20 μl of sample solution were injected using the autosampler and eluted at 1 ml/min with a linear gradient of 100 to 500 m*M* sodium acetate in 100 m*M* NaOH for 40 min. However, only for the analysis of the change in maltoheptaose along with the lapse of time after the dissolution with 100 m*M* NaOH, the linear gradient of 50 to 250 m*M* sodium acetate in 100 m*M* NaOH for 40 min was used instead to obtain a high resolution of the smaller molecules. The pulse program of the PED was as follows: E_1=0.05 V, t_0 =400 ms; E_2=0.75 V, t_2=200 ms; E_3=0.15 V, t_3=400 ms.

The unit-chain distributions of amylopectin were classified at the inflection points of the elution

profiles into three groups (A-, B1- and B2-chains), i.e., the maltooligosaccharides with their DP from 6 to 18 were defined as the A-chain, the DP from 19 to 34 as the B1-chain, and the B2-chain corresponds to a DP from 35 to their retention time at 33.0 min for the reduced samples or at 34.0 min for the non-reduced ones. The peak-area ratios (%) of the three groups were calculated using a work station with the AI-450 chromatography system (Dionex, CA, USA).

3. Results and discussion

3.1. The effect of concentration of sodium hydroxide solution on solubilization of amylopectin isoamylolyzates

The HPAEC elution profiles of amylopectin isoamylolyzates, which are directly dissolved in 100 m*M* NaOH or first with 400 m*M* NaOH and then finally diluted to prepare the sample solution in 100 m*M* NaOH with distilled water, are shown in Fig. 1. The dissolution of amylopectin isoamylolyzate was incomplete by directly adding 100 m*M* NaOH. Especially, the solubility of the B2-chains with 100 m*M* NaOH (Fig. 1b) was much smaller than that with 400 m*M* NaOH (Fig. 1a); the peak-area ratios of the B2-chains showed a large difference between 400 m*M* NaOH (13.1%) and 100 m*M* NaOH (11.1%). Therefore, it was difficult to dissolve the lyophilized isoamylolyzate with the mild alkaline solvent for chain-length distribution analysis of the amylopectin, even though the mild alkaline method is effective in repressing the alkaline degradation of several oligosaccharides as reported by Wang and Zopf [7].

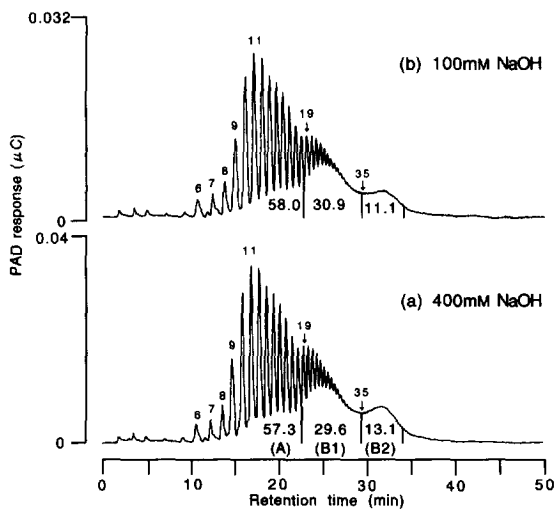

Fig. 1. HPAEC elution profiles of non-reduced isoamylolyzates of wheat amylopectin dissolved with (a) 400 m*M* NaOH and (b) 100 m*M* NaOH. Numbers above the peaks indicate the degree of polymerization of maltooligosaccharides. Values below each elution profile indicate the peak-area ratio (%) of the A-, B1- and B2-chains of amylopectin. Chromatographic conditions: CarboPac PA-1 (250×4 mm I.D.) with a CarboPac PA-1 guard column; eluent A, 100 m*M* NaOH; eluent B, 100 m*M* NaOH containing 500 m*M* CH_3COONa; gradient program, 20% eluent B at 0 min, 100% at 40 min; flow-rate, 1 ml/min; detector, PED.

3.2. The effect of reduction of standard maltooligosaccharides and amylopectin isoamylolyzates with sodium borohydride

The change in the HPAEC elution profiles of maltoheptaose and amylopectin isoamylolyzates along with respect to time after the dissolution of the lyophilized samples with 100 m*M* NaOH are shown in Fig. 2 and Fig. 3, respectively. In Fig. 2, along with the lapse of time, the maltoheptaose was indicated to change into other molecules with retention times shorter than that of maltoheptaose. Most of the molecules could be identified using the standard maltooligosaccharides as the smaller maltooligosaccharides and their epimers as indicated in Fig. 2. The shift in maltoheptaose to the smaller maltooligosaccharides was considered as the result of the epimerization and the degradation of maltooligosaccharides in alkaline solution as reported by Koizumi et al. [6], Niemann and Saenger [8] and Whistler and BeMiller [11]. As shown in Fig. 3, the change in the elution profile of maltooligosaccharides along with time after the dissolution was also observed for the amylopectin isoamylolyzate samples which were dissolved with 400 m*M* NaOH for 30 min and then diluted with deionized water to provide the sample solution in 100 m*M* NaOH. The degradation and epimerization of maltooligosaccharides in amylopectin isoamylolyzate were typically shown by the increase in the peaks of the degradation product which eluted from 1.5 to 4.0 min and the increase in the number of epimeric

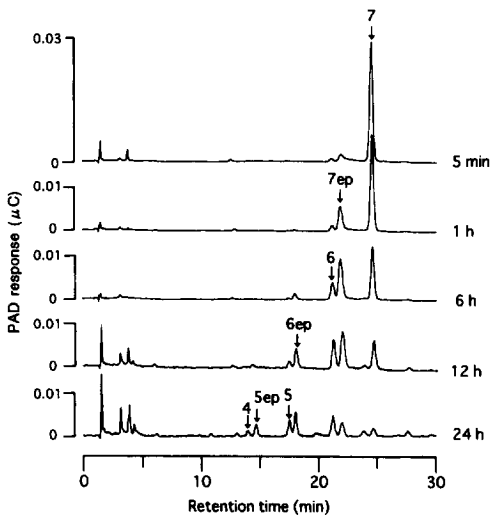

Fig. 2. HPAEC elution profiles of non-reduced maltoheptaose injected at 5 min, 1 h, 6 h, 12 h and 24 h after the dissolution with NaOH. Numbers above the peaks (4–7) indicate the degree of polymerization of maltooligosaccharides. Each peak designated as 5ep, 6ep and 7ep correspond to epimerized oligosaccharides. Chromatographic conditions: gradient program, 10% eluent B at 0 min, 50% at 40 min. Other conditions as stated in Fig. 1.

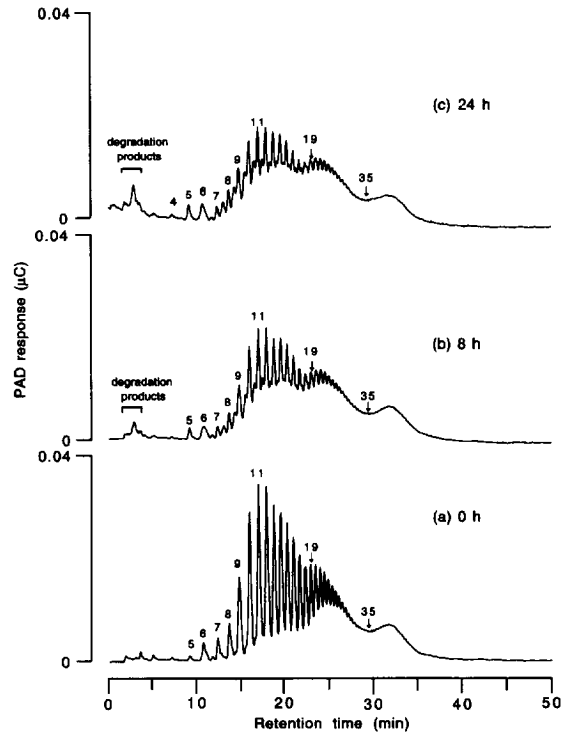

Fig. 3. HPAEC elution profiles of non-reduced isoamylolyzates of wheat amylopectin dissolved at (a) 0 h, (b) 8 h and (c) 24 h after the dissolution with NaOH. Chromatographic conditions as stated in Fig. 1.

peaks which were observed as the shoulder peaks, respectively. From these results, it was considered important for the chain-length distribution analysis of amylopectin to avoid the alkaline degradation and epimerization of maltooligosaccharides in amylopectin isoamylolyzate because of the fragility of maltosaccharides in alkaline solution.

In order to avoid the degradation and epimerization of maltooligosaccharides in alkaline solution, the effect of the reduction of maltooligosaccharides with sodium borohydride was investigated. The HPAEC elution profiles of reduced isoamylolyzates of amylopectin at 0 and 24 h after the dissolution in NaOH are shown in Fig. 4a and b. Neither the degradation nor epimerization of maltooligosaccharides was observed even 24 h after the dissolution. Also, the peak-area ratios of the three groups, which are denoted as the A-, B1- and B2-chains of amylopectin, did not vary with time. Another influence of the reduction of maltooligosaccharides was observed in the shortening of the retention time of each maltooligosaccharide, i.e., non-reduced maltoheptaose was eluted at 12.3 min in this elution program, but it shifted to 8.3 min when it was reduced with sodium borohydride. The reduced standard maltooligosaccharides were also confirmed to be stable in 100 m*M* NaOH and their retention times were shortened.

Based on these results, the reduced maltooligosaccharides, which are converted to the corresponding alditols of the reducing groups, are considered to be stable in NaOH solution, though their retention times were shorter than those of the non-reduced ones. Therefore, the reduction of isoamylolyzates made the chain-length distribution analysis of amylopectin simple and reproducible, and then permitted the use of the autosampler which facilitated the analysis of a large number of samples under the same HPAEC conditions.

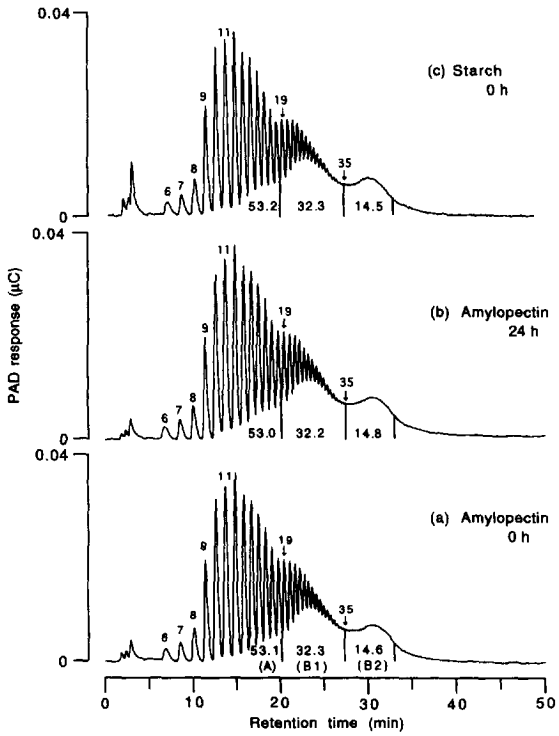

Fig. 4. HPAEC elution profiles of reduced isoamylolyzates of wheat amylopectin at (a) 0 h and (b) 24 h after the dissolution with NaOH and (c) reduced isoamylolyzate of wheat starch. Chromatographic conditions as stated in Fig. 1.

3.3. The influences of the use of starch instead of amylopectin for the analysis of the chain-length distribution of amylopectin

The HPAEC elution profile of the reduced isoamylolyzate of starch is also shown in Fig. 4c in addition to those of the reduced isoamylolyzates of amylopectin (Fig. 4a and b). There was little difference both in the elution profiles and the peak area ratios of the three groups between the reduced isoamylolyzate of amylopectin and that of starch. Amylose, which is the other α-1,4-glucan contained in starch, therefore, was considered to be of little influence on the chain-length distribution analysis of amylopectin. This might be due to the fact that there are very few short chains of amylose as short as those of amylopectin, in addition to the relatively small amount of amylose itself (ca. 20–30%) in starch. It is postulated that the long chains in amylose isoamylolyzate had a very low response to a pulsed amperometric detector. It was, therefore, difficult to detect their peaks, which mainly eluted after 40 min, in the sample of starch isoamylolyzate.

4. Conclusion

The use of starch isoamylolyzate instead of amylopectin isoamylolyzate was found to permit analysis of the chain-length distribution of amylopectin without fractionation of amylopectin from starch and allowing the screening of high-quality wheats having preferable starch for food processing from large numbers of wheat cultivars and breeding lines.

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