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Note

A novel enzymic determination of maltose

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Abstract

A novel enzymic determination of maltose with four enzymes (a new enzyme, maltose 1-epimerase [EC 5.1.3.-], maltose phosphorylase [EC 2.4.1.8], β-phosphoglucomutase [EC 5.4.2.6], and glucose-6-phosphate dehydrogenase [EC 1.1.1.49]) is described. Maltose was rapidly and quantitatively determined within about 2 min by means of maltose 1-epimerase. The standard curve was linear up to 1.5 μmol/mL. The within-run and between-run studies gave precision (CV) values of $<2.0\%$ and $<3.0\%$, respectively. No significant interferences by mono- and disaccharides were observed with the proposed method under this study. There was a good correlation ($r=0.997$) between the results obtained by the enzymic and HPLC methods. This method fulfills the need for an accurate, specific and simple assay of maltose, and it is less time consuming than HPLC and enzymic methods previously reported. © 2000 Elsevier Science Ltd. All rights reserved.

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Maltose (4-*O*-α-D-glucopyranosyl-D-glucose) is mainly used as a sweetening agent, and it is commonly present in various fermented foods and decomposition products of starch by amylases. Maltose has been determined by high-performance liquid chromatographic (HPLC) methods [1,2], and improved HPLC methods available for the analysis of sugar profiles, including maltose in high carbohydrate content foods have recently been demonstrated [3,4]. In addition various enzymic methods have been reported because these methods do not require large, expensive apparatus and are appropriate for use in a large number of analyses of maltose. Two enzymic methods, the α-glucosidase [EC 3.2.1.20]/glucose oxidase [EC 1.1.3.4]-system [5] and the α-glucosidase/hexokinase [EC 2.7.1.1]/glucose-6-phosphate dehydrogenase [EC 1.1.1.49] systems [6], have been developed for the determination of maltose, but these are not acceptable because the α-glucosidase can cleave various other disaccharides (sucrose, lactose, etc.) and some oligosaccharides as well as maltose. Although another method, the maltose phosphorylase [EC 2.4.1.8]/glucose oxidase system [7], is highly specific for determining maltose, it is obviously time consuming for the complete reaction because maltose exits as an equilibrium mixture of α and β anomers in an aqueous solution, and maltose phosphorylase has strict stereospecificity for α-maltose [8]. In a general way, the above systems have a disadvantage in requiring a sample blank which might contain glucose

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that would react in the presence of hexokinase or glucose oxidase. A sugar-oxidizing enzyme with broad substrate selectivity, such as oligosaccharide dehydrogenase, has also been reported to be advantageously used for the analysis of sugars containing maltose or oligo- and polysaccharides in a biosensor format [9,10].

Recently we found that *Lactobacillus brevis* IFO 3345 produced a new enzyme, maltose 1-epimerase [EC 5.1.3.-], which is capable of efficiently catalyzing the interconversion of α- and β-maltose [11]. Furthermore, two thermostable enzymes, maltose phosphorylase from *Enterococcus hirae* IFO 3181 [12] and β-phosphoglucomutase [EC 5.4.2.6] from *Lactococcus lactis* subsp. *cremoris* IFO 3427 [13], were also investigated by us. The three enzymes would be suitable for the system of maltose determination because these have their optimum pH values around pH 7.0, with no significant loss of activity on incubation at 37°C for at least 1 week. In order to establish a rapid and convenient determination of maltose, we devised a simple enzymic endpoint assay with the above-mentioned three enzymes and glucose-6-phosphate dehydrogenase. Our method made it possible to quantitate maltose using this reaction system (Scheme 1), which did not need a sample blank.

The basic measurement is the increase of NADPH is measured by the change in extinction at 340 nm, which is proportional to the amount of maltose present.

1. Experimental

Materials.—Maltose monohydrate and D-glucose 1,6-diphosphate were purchased from Nacalai Tesque Inc., Japan and Boehringer Mannheim Corp., Germany, respectively. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* and α-amylase [a mixture of both α-amylase (2.1 U/mg solid) and β-amylase (2.9 U/mg solid)] from barley malt were respectively from Oriental Yeast Co. Ltd., Japan and from Sigma Chemical Co., USA. All other chemicals were of the highest grade available. Maltose 1-epimerase, maltose phosphorylase and β-phosphoglucomutase were products of Kikkoman Corp. and are commercially available.

Preparation of reagents.—A 1.0 mM maltose standard solution (distilled water) was freshly prepared. The reagent mixture was prepared to contain 10 U/mL maltose 1-epimerase, 10 U/mL maltose phosphorylase, 3.0 U/mL β-phosphoglucomutase, 10 U/mL glucose-6-phosphate dehydrogenase, 0.3 mM D-glucose 1,6-diphosphate, 1.2 mM $NADP^+$, 20 mM KCl, 2.0 mM $MgSO_4$, 0.25% Triton X-100 in 50 mM phosphate buffer (pH 7.0).

Procedure for the determination of maltose.—Maltose was enzymically assayed by the procedure described as follows, using a Hitachi U-2000A spectrophotometer (Hitachi Ltd., Japan). To the reagent mixture (0.9 mL) was added a 0.1 mL sample (1.0 mM maltose standard solution or distilled water). After reaction for 3 min at 37 °C, the absorbance of

$$\beta\text{-Maltose} \xrightarrow{\text{maltose 1-epimerase}} \alpha\text{-Maltose}$$

$$\alpha\text{-Maltose} + \text{Phosphate} \xrightarrow{\text{maltose phosphorylase}}$$

$$\alpha\text{-D-Glucose} + \beta\text{-D-Glucose 1-phosphate}$$

$$\beta\text{-D-Glucose 1-phosphate} \xrightarrow{\beta\text{-phosphoglucomutase}} \beta\text{-D-Glucose 6-phosphate}$$

$$\text{D-Glucose 6-phosphate} + \text{NADP}^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}}$$

$$\text{6-Phospho-D-gluconate} + \text{NADPH} + \text{H}^+$$

Scheme 1. Reaction system for the determination of maltose.

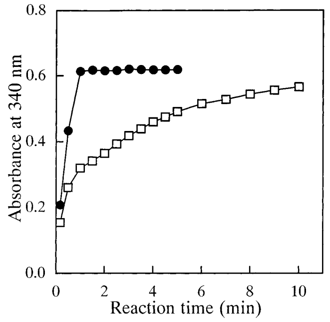

Fig. 1. Reaction curves for the determination of maltose. The reaction curves of the maltose standard solution (1.0 µmol/mL) were obtained in the presence (●) and absence (□) of maltose 1-epimerase as described in the Experimental section.

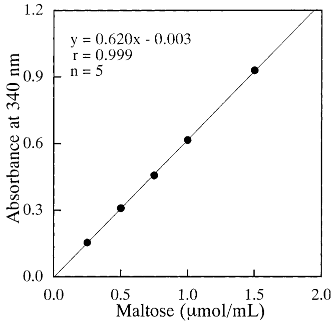

Fig. 2. Standard curve obtained with various dilutions of maltose solution.

the reaction mixture at 340 nm against distilled water was measured.

The amount of maltose was calculated by the following formula:

$$\text{Maltose } (\mu\text{mol/mL}) = \frac{(E_s - E_b)}{(E_{st} - E_b)} \times 1.0$$

where E_s, E_{st}, and E_b are absorbances of the reaction mixtures of the sample, the maltose standard, and the distilled water, and 1.0 represents the concentration of the maltose standard solution (µmol/mL).

HPLC method.—To compare with the proposed enzymic method, maltose was also analyzed by an HPLC method. The operating conditions were as follows: column, TSKgel Amido-80 (4.6 mm i.d. × 250 mm, Tohso Co., Japan); solvent, 82:18 acetone–water; flow rate, 1.0 mL/min; column temperature, 80 °C; injection volume, 20 µL. The effluent was monitored using a refractive index detector (a Shodex RI-71, Showa Denko K.K., Japan). The detection limit for this method was about 2 µmol/mL, and the time required for an individual run was 30 min.

Preparation of maltose samples.—Samples containing various maltose concentrations were prepared as follows: to 4% soluble potato starch solution (100 mL) in 0.1 M acetate buffer (pH 5.5) was added 2 mL barley malt α-amylase solution (100 mg/mL). Several samples containing maltose thus formed were taken from the mixture during incubation at 37 °C for 0–120 min and then heated for 3 min in a boiling water bath to stop the hydrolyzing reaction. The supernatants of the samples were collected by centrifugation and stored at −20 °C until analysis.

2. Results and discussion

Assay conditions.—It was reported by Kamogawa et al. [7] that the quantitative determination of maltose using maltose phosphorylase took almost 120 min because of the enzyme's strict specificity for α-maltose. To evaluate the optimal reaction time for the determination of maltose, the time-course for phosphorolysis of maltose by maltose phosphorylase was examined in the presence or absence of maltose 1-epimerase as shown in Fig. 1. Although the reaction in the presence of maltose 1-epimerase went to completion within about 2 min, the reaction in the absence of the epimerase was not quite completed even after a lapse of 10 min. It was demonstrated that maltose could be rapidly and quantitatively determined by the developed novel method by means of maltose 1-epimerase.

Reliability of the measurement.—The linearity of a calibration curve constructed from aqueous maltose standard solutions was assessed (Fig. 2). When net absorbance change was plotted versus maltose concentration, the relation was linear up to 1.5 µmol/mL, the linear regression equation being $y = 0.620x - 0.003$ µmol/mL ($n = 5$, correlation coefficient 0.999). The lowest detection for this assay was about 0.02 µmol/mL. To determine within-run and between-run precision (CV values), two maltose solutions (0.20 and 1.0 µmol/mL) were assayed five times each. The within-run precision (CV) for maltose solutions was found to be $< 2.0\%$, and the between-run

Table 1
Effect of substances on the maltose determination

Substances (10 μmol/mL)	Apparent maltose (%)	Substances (10 μmol/mL)	Apparent maltose (%)
None	100	sucrose	102
D-Glucose	99	lactose	100
D-Fructose	101	maltotriose	98
D-Galactose	101	maltopentaose	98
α,α-Trehalose	101	maltoheptaose	98
Cellobiose	100		

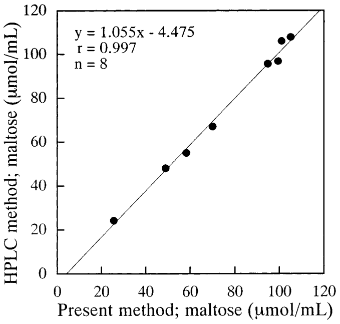

Fig. 3. Correlation between maltose concentrations in the samples obtained by the present and HPLC methods.

precision (CV) for daily maltose analyses of aqueous solutions was $< 3.0\%$. The precision of this method was satisfactory and was similar to those of many enzymic methods applied to food chemistry and clinical chemistry. The effect of several substances such as monosaccharides (D-glucose, D-fructose and D-galactose), disaccharides (α,α-trehalose, cellobiose, sucrose and lactose) and oligosaccharides (maltotriose, maltopentaose and maltoheptaose) was examined on the determination of maltose (Table 1). Although the maltose standard (0.1 μmol/mL of reaction mixture) was assayed with and without addition of the substances at the concentrations of at least up to 10 μmol/mL of reaction mixture, these did not significantly affect the present method. Therefore, it seemed that the assay system coupled with four enzymes was reliable for the determination of maltose without any significant interference from various saccharides.

Comparison of the enzymic method with an HPLC method.—The enzymic method was compared with an HPLC method using eight samples containing various maltose concentrations prepared from potato soluble starch, as shown in Fig. 3. A good correlation ($r = 0.997$) was obtained between the two methods and the regression equation for these data was $y = 1.055x$-4.475 μmol/mL, indicating that the proposed method fulfilled the need for an accurate and specific assay of maltose, and that it was very simple to run as compared with previous HPLC methods.

In conclusion, it is likely that the enzymic method proposed here will be valuable and useful for the determination of maltose in food chemistry and in clinical chemistry. The method would make it possible to carry out multiple analyses using a microtitre plate or an automatic analyzer.

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