A novel enzyme, maltose 1-epimerase from *Lactobacillus brevis* IFO 3345

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Abstract A novel enzyme, maltose 1-epimerase (MER), that catalyzes the interconversion of α and β anomers of maltose was found in a cell-free extract of *Lactobacillus brevis* IFO 3345, and MER was purified to homogeneity from the crude extract. The M_r of the enzyme was estimated to be 43,000 and 45,000 by HPLC gel filtration and SDS-PAGE, respectively. It showed optimum activity at pH 6.5–7.0. This novel enzyme catalyzed the conversion of β-maltose more effectively than disaccharides such as α-lactose and β-cellobiose, whereas the relative velocities for β- and α-D-glucose were about one forth of that for β-maltose.

Key words: Maltose 1-epimerase; Aldose 1-epimerase; Interconversion; *Lactobacillus brevis* IFO 3345

1. Introduction

Aldose 1-epimerase (mutarotase, EC 5.1.3.3) is known to catalyze the interconversion of α and β anomers of certain monosaccharides including D-glucose. It has been reported that the epimerases from various sources such as *Penicillium notatum*, *Escherichia coli*, green pepper and bovine kidney are highly specific for certain monosaccharides but show negligible activity toward a disaccharide such as maltose [1,2]. Although the rate of spontaneous anomerization of maltose is shown to be slower than that of D-glucose [3], there has been no report of an epimerase that can act on maltose specifically. Recently, we found that *Lactobacillus brevis* IFO 3345 produced an intracellular enzyme capable of catalyzing the interconversion of α- and β-maltose efficiently. We have suggested that this enzyme might be a new epimerase (systematic name: maltose 1-epimerase), classified as EC 5.1.3.-. This paper describes the purification and some properties of this novel enzyme.

2. Materials and methods

2.1. Materials

β-Maltose·H_2O and maltose phosphorylase were purchased from Wako Pure Chemical Industries, Ltd., Japan. α-Maltose (α,β(3:1)-complex of anhydrous maltose; $[\alpha]_D = +160°$, reported in [4]) was obtained from Hayashibara Biochemical Laboratories, Inc., Japan. β-D-Glucose was from Sigma Chemical Co., USA. All other chemicals and enzymes were of the highest grade generally available.

2.2. Culture conditions

Lactobacillus brevis IFO 3345 were cultured without aeration at 27°C for 20 h in a 1-ton tank containing 900 liters of the medium (1.0% maltose, 1.0% yeast extract, 2.0% polypepton, 1.0% sodium acetate, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.0002% $MnCl_2 \cdot 4H_2O$, pH 7.0).

2.3. Enzyme assay

The enzyme activity was measured by two methods as follows. Method B was used as a routine assay, unless otherwise stated.

Method A. A reaction mixture (10 ml: 50 mM HEPES-NaOH buffer, pH 7.0) containing 1.0% β-maltose (292 μmol) in the presence or absence of an enzyme sample at 25°C was rapidly introduced into the polarimeter tube keeping constant temperature (Model DIP-360, Nippon Bunko Kogyo Co.) with a Na lamp. The spontaneous plus enzyme-catalyzed and spontaneous velocities were obtained by measuring the increase of optical rotation (millidegree/min), and the enzyme-catalyzed portion of the reaction was calculated by difference. In this assay, the conversion of 1 μmol of β-maltose to α-maltose corresponds to 1.64 millidegrees as the change of optical rotation [5].

Method B. α-Maltose formation was determined under the following conditions because of the specificity of maltose phosphorylase for α-maltose [6]. A reaction mixture (1.0 ml: 50 mM HEPES-NaOH buffer, pH 7.0) containing 0.3 μmol of β-maltose, 11 U of maltose phosphorylase, 40 μmol of phosphate, 100 U of mutarotase, 90 U of glucose oxidase, 10 U of peroxidase, 10 μmol of phenol and 0.5 μmol of 4-aminoantipyrine in the presence or absence of an enzyme sample was incubated at 25°C, and the increase of absorbance (OD/min) at 505 nm in the spontaneous plus enzyme-catalyzed and spontaneous reactions was measured and the enzyme activity was calculated by difference.

One working unit of enzyme activity is defined as the amount of enzyme which catalyzes the conversion of 1 μmol of β-maltose to α-maltose per min under the conditions of Method A. The increase of absorbance per min, 0.30, under the conditions of Method B corresponded to 1 working unit.

2.4. Enzyme purification

The cells were harvested from 900 liters of culture broth with a hollow-fiber concentrating apparatus (Microza; Asahi Kasei Kogyo Co.). To the cell suspension (27 l), 10 l of 10 mM phosphate buffer (pH 7.5) containing 1.0% Triton X-100, 0.5% lysozyme and 0.5 M ammonium sulfate [$(NH_4)_2SO_4$] were added. After standing for 2 h at 35°C, 1.0 l of 10% polyethyleneimine solution (pH 7.5) was added dropwise to the mixture, and then the precipitation formed was removed by filtration. The filtrate was concentrated to 12.8 l by ultrafiltration, and 1280 ml of the concentrate were used for purification. The concentrate was diluted 3 times with 10 mM phosphate buffer, pH 6.5 (Buffer A) and applied to a QAE-Sephadex A-50 column (13 × 40 cm) equilibrated with Buffer A containing 0.2 M KCl. The enzyme was eluted with Buffer A containing 0.4 M KCl, and the active fractions were concentrated to 320 ml by ultrafiltration. The concentrate was diluted 3 times with Buffer A and subjected to rechromatography with a QAE-Sephadex A-50 column (8 × 22 cm) using a linear concentration gradient of KCl (0.2 to 0.4 M) in Buffer A. The eluted active fractions were concentrated to 42 ml by ultrafiltration. To the concentrate, $(NH_4)_2SO_4$ was added up to a final concentration of 20% saturation. The enzyme was applied to a phenyl-Sepharose CL-4B column (2.5 × 40 cm) equilibrated with Buffer A containing $(NH_4)_2SO_4$ (20% saturation). The enzyme was eluted with a reverse linear concentration gradient of $(NH_4)_2SO_4$ (20 to 0% saturation) in Buffer A. The active fractions were concentrated to 30 ml by ultrafiltration and then dialyzed against 2 mM phosphate buffer (pH 6.8). The enzyme was applied to a hydroxylapatite column (2.5 × 16 cm) equilibrated with 2 mM phosphate buffer (pH 6.8). The column was eluted with a linear concentration gradient of phosphate buffer, pH 6.8 (2 to 50 mM). The active fractions were concentrated to about 2 ml by ultrafiltration. The enzyme was put on a Sephadex G-200 column (2.5 × 95 cm) equilibrated with Buffer A containing 0.1 M NaCl and filtered with the same buffer. The active fractions (48 ml) were combined.

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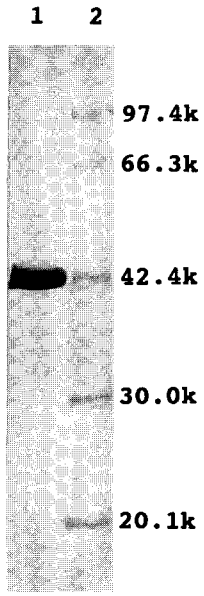

Fig. 1. SDS-PAGE of the purified MER. The gel was stained with Coomassie brilliant blue (R-250) and destained in 7% acetic acid. Lane 1, purified enzyme (about 10 μg); lane 2, marker proteins.

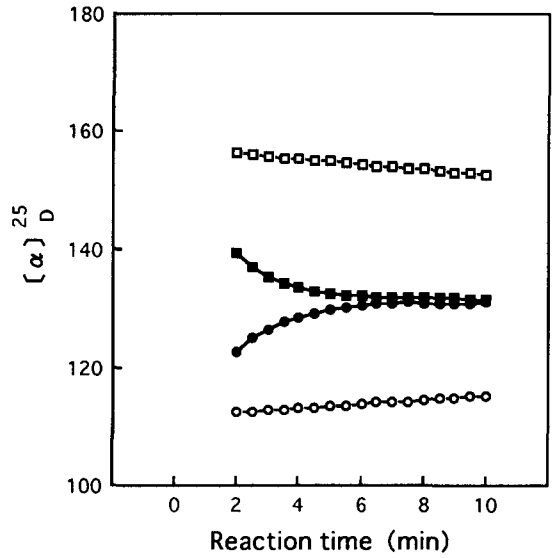

Fig. 2. Equilibrium studies by MER. Details of the polarimetric assay conditions are described in Section 2. The specific rotations were determined with α-maltose [α,β(3:1)-complex] in the absence (□) and presence (■) of 60 U of MER, and with β-maltose in the absence (○) and presence (●) of 60 U of MER.

2.5. Other methods

The protein was measured with a Bio-Rad Protein Assay kit (standard protein: bovine plasma gamma globulin). SDS-PAGE was done by the method of Laemmli [7]. HPLC gel filtration on TSKgel G3000SW$_{XL}$ was carried out by the method of Fukano and Komiya et al. [8].

3. Results and discussion

A novel enzyme, MER, was purified up to homogeneity 389-fold with a yield of 33% from the cell-free extract (Table 1), and its specific activity was 1710 U/mg protein. The purified enzyme gave a symmetric single peak at the final stage. The M_r of the native enzyme was estimated to be 43,000 from the retention time of HPLC gel filtration with TSKgel G3000SW$_{XL}$. From the mobility of SDS-PAGE, the M_r of the enzyme protein monomer was found to be 45,000 (Fig. 1). Thus, MER appeared to have a monomeric structure.

Although the enzyme showed optimum activity around pH 6.5–7.0 using Method A, more than 80% of the maximum activity was retained in the range of pH 5.0 to 8.0. The enzyme was stable over the range of pH 5.0 to 8.5 on incubation at 30°C for 30 min. The optimum temperature of the enzyme was estimated to be about 40°C. When the enzyme was incubated at 20–75°C for 10 min in 50 mM phosphate buffer (pH 7.0), the remaining activity was 100% of the original level at 45°C, and about 50% at 60°C. The activity was almost completely inhibited by Hg^{2+} and Cu^{2+} ions, while it was insensitive to metal-chelating reagents and sulfhydryl-group blocking agents. The K_m value was estimated to be 2.2 mM from the Lineweaver–Burk plot.

The catalytic action of MER was investigated by Method A as shown in Fig. 2. Both specific rotations of α-maltose [α,β(3:)-complex] and β-maltose in the reaction mixtures with 60 U of MER shifted to around + 131° within about 6 min of incubation, indicating the interconversion of α and β anomers of maltose until the equilibrium was attained. The spontaneous anomerization of α-maltose or β-maltose was also observed to occur gradually in the absence of MER. No hydrolytic product of maltose such as D-glucose was detected in the reaction mixtures by HPLC using a TSKgel Amido 80 column as described preciously [9]. From the above results, it was concluded that maltose 1-epimerase catalyzed the following reaction.

α-Maltose ⟷ β-maltose

Table 1
Purification of maltose 1-epimerase from *Lactobacillus brevis* IFO 3345

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Fold
Crude extract[a]	31600	139000	4.4	100	1
1st QAE-Sephadex A-50	11300	124000	11.0	89	3
2nd QAE-Sephadex A-50	2590	76900	29.7	55	7
Phenyl-Sepharose CL-4B	764	65300	85.5	47	19
Hydroxylapatite	37.3	55200	1480	40	336
Sephadex G-200	26.4	45200	1710	33	389

[a] Corresponding to 90 liters of the culture broth.

Table 2
Substrate specificity of maltose 1-epimerase

Substrate	Δ Optical rotation (millidegree/min)		Relative velocity (%)
	Spontaneous plus enzyme-catalyzed	Spontaneous	
β-Maltose	6.75	3.71	100
α-Lactose	−5.95	−5.73	5
β-Cellobiose	3.56	3.44	4
β-D-Glucose	9.82	8.05	27
α-D-Glucose	−1.61	−1.33	26

The reactions were done with various substrates under the assay conditions of Method A in the presence or absence of 1.85 U of MER. The relative velocities were calculated from the following formula.

$$\frac{(\Delta OR_{catalyzed}/\Delta OR_{spontaneous} - 1)\ \text{(for sugar)}}{(\Delta OR_{catalyzed}/\Delta OR_{spontaneous} - 1)\ \text{(for } \beta\text{-maltose)}} \times 100$$

$\Delta OR_{catalyzed}$: Δ Optical rotation (spontaneous plus enzyme-catalyzed).
$\Delta OR_{spontaneous}$: Δ Optical rotation (spontaneous).

Table 2 shows the relative velocities for various substrates by MER. The enzyme catalyzed the conversion of β-maltose more effectively than disaccharides such as α-lactose and β-cellobiose, whereas the relative velocities for β- and α-D-glucose were about one forth of that for β-maltose. This substrate specificity was quite distinct from those of aldose 1-epimerases from *Penicillium notatum* (relative velocities; D-glucose/maltose = 100%/3%), *Escherichia coli* (100%/2%), green pepper (100%/6%) and bovine kidney (100%/0%). The enzyme also differed from cellobiose 2-epimerase (EC 5.1.3.11), because it epimerizes the configuration at carbon-2 of the reducing moiety of cellobiose [10]. In 'Enzyme Nomenclature', no epimerase capable of acting on maltose specifically among saccharides and the derivatives has been reported. Consequently, it is obvious that this enzyme is a new epimerase, classified as EC 5.1.3.-, whose systematic name should be maltose 1-epimerase.

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