Characterization of Extracellular β-d-Galactosidase from *Fusarium moniliforme* Grown in Whey†

B. J. MACRIS‡ and P. MARKAKIS*

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

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Extracellular lactase (β-d-galactosidase, EC 3.2.1.23) was prepared as an ethanol precipitate from a culture of *Fusarium moniliforme* grown on whey. The enzyme functioned optimally at pH 3.8 to 5.0 and at 50 to 60°C on both o-nitrophenyl-β-d-galactopyranoside (ONPG) and lactose. The activation energy of the enzymic hydrolysis of ONPG and lactose in the range of 20 to 55°C was 8,500 and 7,200 cal (ca. 3.57 × 10^4 and 3.02 × 10^4 J)/mol, respectively. The *Km* values were 4.4 and 12.4 mM for ONPG and lactose, respectively. At optimum pH, the enzyme lost half of its activity when it was heated at 50°C for 6 h; at the same pH, the loss was only 5% when the enzyme was heated at 37°C for 6 h. At optimum conditions, 50% of the lactose in whey was hydrolyzed by 10 U of this enzyme in 50 h.

β-d-Galactosidase catalyzes the hydrolysis of lactose to glucose and galactose. Whereas lactose is important as a milk nutrient and a fermentation substrate in many milk products, it also presents problems as a substance easily crystallizing in condensed and frozen milk products, a pollutant when untreated whey is discharged, and a sugar poorly tolerated by many people (11). The interest of many investigators (1, 4, 8–10, 12, 15, 16) in finding suitable sources of lactase for facilitating the hydrolysis of this sugar is understandable.

Certain bacteria (e.g., *Streptococcus thermophilus*, Lactobacillus helveticus) (8, 10), yeasts (e.g., *Saccharomyces lactis*, *Saccharomyces fragilis*) (5, 12), and molds (e.g., *Aspergillus niger*, *Aspergillus foetidus*, *Aspergillus oryzae*) (2, 8, 13, 15, 16) (A. Mustranta, E. Karvonen, and M. Linko, Abstr. VIth Int. Ferm. Syrup. London, Ontario, Canada, 1980) are known lactose producers. The search continues for organisms yielding large quantities of lactase, which can be easily extracted and which possesses characteristics (pH and temperature optima, resistance to product inhibition, etc.) suitable for the intended applications. *Fusarium moniliforme* is a mold which produces extracellular lactase as well as protein of good nutritional quality (3, 6). This study deals with certain physical and chemical characteristics of the lactase produced by *F. moniliforme*.

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‡ Present address: Biology division, N. R. C. “Demokritos,” Aghia Paraskevi, Attikis, Greece.

MATERIALS AND METHODS

Enzyme preparation. The strain of *F. moniliforme* previously used for microbial protein production was employed (3, 6). The mold was grown in whey under optimum conditions as reported elsewhere. The mycelium was removed from the culture medium by centrifugation (5,000 × g for 30 min), and the supernatant was passed through a membrane filter with a 1.2-μm pore size. The enzyme was precipitated from the filtrate at 4°C by the addition of ethanol to a final concentration of 70% by volume. The precipitate was freeze-dried and used in the subsequent work. The yield in freeze-dried powder was 7.5 mg/ml of filtrate, and the yield in enzyme activity (of the powder) was 2 U/ml of filtrate.

Enzyme assay. Lactase activity was determined by using as the substrate either o-nitrophenyl-β-d-galactopyranoside (ONPG) or lactose. A 1-ml sample of enzyme solution was incubated for 10 min at 60°C with 4 ml of 0.1 M acetate buffer (pH 5.0) containing either 3.0 mM ONPG or 30.0 mM lactose. Denatured enzyme (heated at 100°C for 10 min) was used in the blank. When ONPG was used as the substrate, the reaction was stopped by adding 1 ml of a 30% Na2CO3 solution (wt/vol), and the liberated o-nitrophenol was measured photometrically at 420 nm. The liberated glucose was estimated with glucin carb reagent (ISV T Scavo, Divisioni Diagnostici, Siena, Italy). One unit of lactase activity was defined as the amount of enzyme required to liberate 1 nmol of o-nitrophenol per s under the conditions described above.

Hydrolysis of whey lactose. Commercial acid whey powder was dissolved in water to make a 5% lactose solution (wt/vol). The pH of the solution was adjusted to 4.3 with concentrated HCl, and the coagulated casein was removed by filtration through Whatman no. 1 paper. The filtrate was incubated with *F. moniliforme* lactase, and the released glucose was...
measured. Lactose was determined colorimetrically (7).

RESULTS AND DISCUSSION

The effect of pH and temperature on lactase activity with ONPG and lactose as the substrates is shown in Fig. 1 and 2. The optimum pH appears to be between 3.8 and 5.0, and the optimum temperature appears to be between 50 and 60°C. An Arrhenius plot (the log of the reaction rate versus the reciprocal of the absolute temperature) allows the calculation of an average energy of activation equal to 8,500 and 7,200 cal (ca. 3.57 × 10^4 and 3.02 × 10^4 J)/mol for the enzymic hydrolysis of ONPG and lactose, respectively. These values were lower than that (12,900 cal [ca. 5.42 × 10^4 J/mol]) reported for the lactase of S. fragilis (14).

A Lineweaver-Burk plot (the reciprocal of the reaction rate versus the reciprocal of the substrate concentration) for tests performed at pH 4.3 and 50°C allowed the calculation of K_m values equal to 4.4 and 12.4 mM for ONPG and lactose, respectively, and a V_max equal to 5.5 nmol/s per U and 0.5 mmol/s per U, respectively.

The thermostability results are shown in Fig. 3. At 37°C, the enzyme is rather stable, as in 6 h it lost only 5% of its activity. At 50°C, the loss of activity was 50% in 6 h. A logarithmic destruction time relationship is apparent from these results.

The properties of a number of commercial microbial lactases are summarized in Table 1.

From these data it can be concluded that the properties of F. moniliforme lactase are not very different from those of other mold lactases.

Data regarding the hydrolysis of lactose in whey by means of the F. moniliforme lactase are shown in Fig. 4 and 5. Figure 4 shows the effect of temperature on this hydrolysis, and Fig. 5 shows the effect of enzyme concentration on hydrolysis. With 10 U of lactase activity, 50% of the whey lactose was hydrolyzed within 50 h of incubation at 55°C. It is apparent that the extra-
cellular F. moniliforme lactase has potential for the commercial conversion of whey lactose to galactose and glucose.

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