# MAMMALIAN β-GALACTOSIDASES

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Bacterial β-galactosidase is an enzyme that has been studied in great detail. However, little is known about the enzyme found in mammalian liver, although it is thought to be associated with lysozymes (Sellinger et al., 1960). The intestinal enzyme has attracted considerable attention because it appears shortly after birth (Maio and Rickenberg, 1960; Doell and Kretchmer, 1962; Alvarez and Sas, 1961; Koldovsky and Chytil, 1965; Huber et al., 1961). In preparations taken from the small intestine of the mouse, it had a pH optimum of 2.8 (Maio and Rickenberg, 1960). Koldovsky and Chytil (1965) have demonstrated that the pH optimum for β-galactosidase is different in various parts of the small intestine of suckling rats. The present study is concerned with the possibility of the existence of multiple types of β-galactosidase in beef and rat liver.

Beef liver that was transported on ice from the slaughter house and rat liver that was removed immediately after the animal was killed were cut into small pieces and homogenized in 2 volumes by weight of ice-cold sucrose (0.25 M) with an all-glass Potter-Elvehjem homogenizer. The homogenates were sonicated for 30 min in a Raytheon sonicator (10 Kc) under cooling and centrifuged at 105,000 X g for 60 min. The clear supernatant was kept frozen until used. The β-galactosidase activity in the preparations was determined after appropriate dilution with o-nitrophenyl- β-D-galactoside as the substrate, according to the method of Lederberg (1950) and as modified by Koldovsky and

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Chytil (1965). Citrate hydrochloric acid buffer was used at pH 2 to 3, sodium acetate buffer at pH 3 to 5.5, and sodium phosphate buffer at pH 5.5 to 8.0.

p-Nitrophenyl-β-D-glucoside and p-nitrophenyl-phosphate were used as substrates to determine the activity of β-glucosidase and of phosphatase. The activity of lactase was followed by the glucose oxidase method ("Glucostat," Worthington).

The equipment for the starch gel electrophoresis of the β-galactosidase has been described by Fine and Costello (1963). A solution of 30 ml 1 M sodium acetate and 18 ml 4 M acetic acid diluted to 1000 ml was used for the well buffer, which was diluted with 3 volumes of distilled water for the tray buffer. The final pH of the buffers was 4.0. A constant current of 30 milliamps (340-380 V) was applied on the gels for 16 to 20 hrs. The β-galactosidases on the gel were identified by staining with 6-bromo-2-naphthyl-β-D-galactoside as substrate, which is an adaptation of the histochemical method of Cohen et al.(1952). The starch gels were cut into thin slices and sprayed with a substrate solution consisting of one volume of the stock substrate solution* and one volume of water (for following the localization at pH 4.0) or with one volume of 0.3 M sodium phosphate buffer (for the detection of the enzyme at pH 7.0). Sodium chloride was added to the substrate solution so that the final concentration of the solution was 1 M. After the gels were incubated for 1 hr, at 37°, the substrate solution was poured off and replaced by a coupling solution prepared beforehand (100 ml water, 20 ml 0.3 M sodium phosphate, pH 7.2, and 100 mg tetrazotized-O-dianisidine) for a period of at least 30 min. The presence of β-galactosidase activity was indicated by a violet or red-violet spot. The excess of the coupling solution was washed out from the gels by water.

The liver β-galactosidases were separated on a Sephadex G-100 column. Three to five ml of crude enzyme preparation from rat or beef liver were ap-

* The stock substrate solution was composed of the following: 10 mg of 6-bromo-2-naphthyl-β-D-galactoside dissolved in 20 ml of 50% methanol by heating to the boiling point and the resulting solution then diluted with 30 ml of water.

plied in a cold room on a column (2 X 38 cm) that had previously been equilibrated with 0.05 M Tris buffer, pH 7.0, containing 0.1 M KCl. The enzymes were eluted in fractions from the column by the same buffer (flow rate approximately 3 ml per hr). The proteins in the eluates were followed by measuring the optical density at 280 mμ. The β-galactosidase activity was determined with buffers of pH 4 and 7 by the above-mentioned method. The molecular weights of the liver β-galactosidases were determined from the crude preparations on a Sephadex G-100 column (2 X 50 cm), according to the method of Andrews (1964). The same buffer was used for the separation experiments, pH 7.2, at room temperature.

The pH dependence of the β-galactosidase activities in the preparations of the rat and of the beef is shown in Fig. 1. The narrow curve shows a sharp

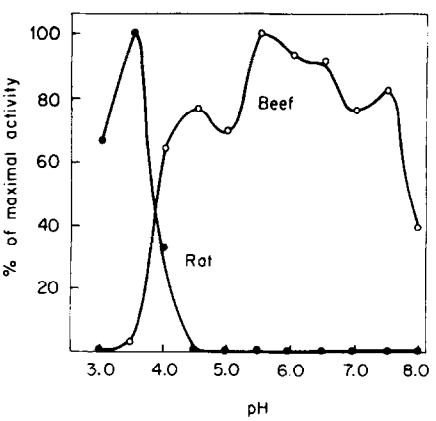


Fig. 1. pH dependence of rat and beef liver β-galactosidase activity. The conditions of the assay are described in the text. Activity is expressed in per cent of maximal activity. ●-●-●: rat liver activity, 0.97 μ moles o-nitrophenyl-β-D-galactoside hydrolyzed by 1 g (wet weight) liver per min, pH 3.5; o-o-o: beef liver activity, 1.97 μ moles/g/1 min, pH 5.5.

maximum for the crude rat liver preparations with the peak of the activity at pH 3.5. In contrast, the curve for the beef preparations is considerably broader with a peak at pH 5.5. The starch gel electrophoresis revealed the presence (Fig. 2) of two enzymatic activities in the beef preparation, one activity with a relative electrophoretic mobility of 1.0 and the other with a

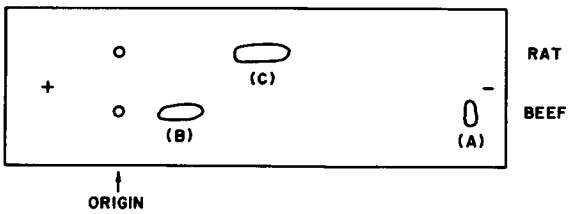


Fig. 2. Starch gel electrophoresis of liver β-galactosidases at pH 4.0. The conditions of the assay are described in the text. A: Activity detectable after incubation with substrate at pH 7.0. B and C: Activity detectable after incubation with substrate only at pH 4.0.

lower relative mobility of 0.18. The slower moving enzyme was detected only by staining at pH 4.0; whereas staining the gels at pH 7.0 showed only the fast moving spot. In the rat preparation only one spot could be detected (relative mobility: 0.43). The rat enzyme was detected only at a lower pH (4.0).

Interestingly, similar results were shown by chromatography on a Sephadex G-100 column. In the rat preparation only one peak for β-galactosidase activity was detected at pH 4.0 near the main peak of the proteins (Fig. 3A). However, two peaks of activity are shown for the beef preparations in Fig. 3B, one peak

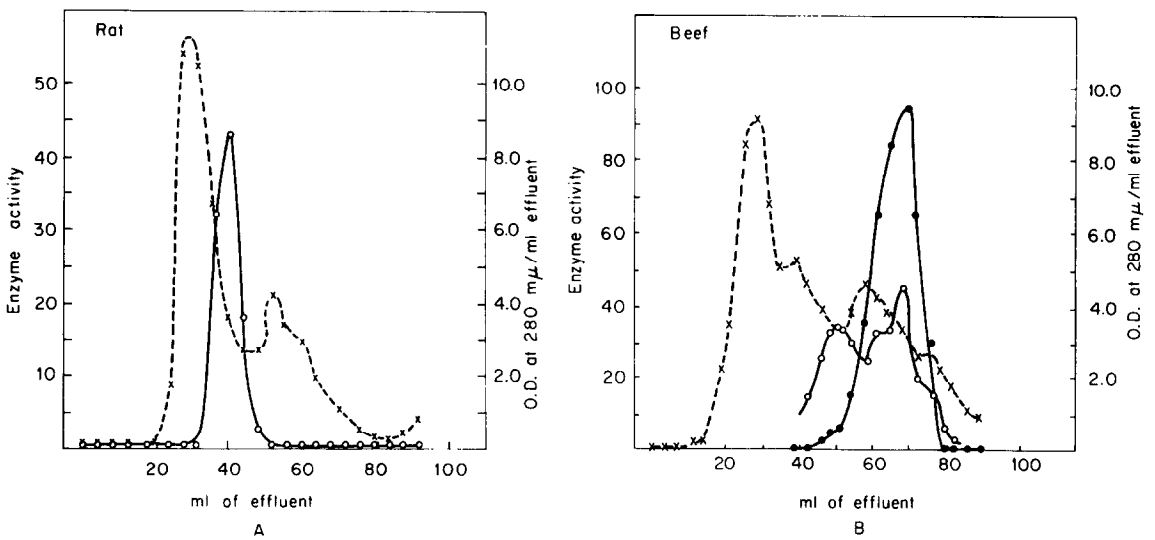


Fig. 3. Separation of the β-galactosidases of rat liver and of beef liver on a Sephadex G-100 column. Enzyme activity was measured at pH 4.0 and is expressed in μmoles X $10^3$ o-nitrophenyl-β-D-galactoside hydrolyzed with 1 ml of eluate per 1 min. A. Rat -------: proteins. B. Beef O—O—O—O: enzymatic activity measured at pH 4.0. ●—●—●—●: enzymatic activity measured at pH 7.0.

with low activity and a pH optimum of 4.0 and the other with higher activity with an optimum close to pH 7.0. When the three enzymatic activities were placed together the rat enzyme, the beef enzyme I, and the beef enzyme II were eluted in sequence from the column. The molecular weights, as determined on a Sephadex G-100 column, are shown in the table. The pH optima for the three

Comparison of β-Galactosidase, β-Glucosidase and Phosphatase Activities in β-Galactosidase Preparations from a Sephadex G-100 Column

| | Rat | Beef I | Beef II |
|---|---|---|---|
| pH optimum | 3.0 | 4.5 | 6.0 |
| Molecular weight* | 127,000 | 85,000 | 43,000 |
| Activity** of: | | | |
| β-galactosidase | 13 | 25 | 93 |
| β-glucosidase | 0 | 19 | 222 |
| Phosphatase | 113 | 47 | 0 |

* Expressed in μmoles X $10^3$ nitrophenol liberated/min/enzyme. The conditions of the assays were the same as for the determination of β-galactosidase activity (Koldovsky and Chytil, 1965).

** Calculated from known molecular weights of enzymes according to the method of Andrews (1964).

enzymes that were separated on the Sephadex column are also shown in the table. Each enzyme has a distinct pH optimum that can be correlated with its molecular weight, as determined from the Sephadex column. The molecular weight of the beef enzyme with a pH optimum at 4.5 appears to be twice that for the beef enzyme with a pH optimum at 6.0. Electrophoresis of the fraction obtained from the Sephadex column showed that only one form of the enzyme was present in each fraction. The electrophoretic mobilities correspond to the pH optima observed in the crude extracts. The rat enzyme with a very acidic pH optimum (3.0) has a molecular weight of 127,000, which is considerably higher than for the two beef enzymes.

Preliminary investigations of the substrate specificity of the fractions

eluted from the Sephadex column, which had the highest β-galactosidase activity, showed that the rat preparation was associated with a high phosphatase activity (at pH 3.0). However, the beef preparation with pH optimum 6.0 has a high β-glucosidase activity at pH 6.0 (see table). No lactase activity was detected in any of the three purified preparations. See also figure 4.

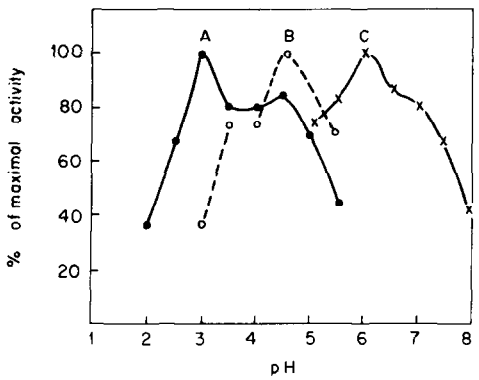


Fig. 4. pH dependence curves of the liver β-galactosidases after separation on a Sephadex G-100 column. Activity is expressed as in Fig. 1. A. Rat enzyme. B. Beef enzyme I. C. Beef enzyme II.

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The bacterial β-galactosidase of Escherichia coli has a pH optimum at 7.3 (Wallenfels, 1962); its molecular weight is estimated to be between 500,000 and 750,000 (Wallenfels and Arens, 1960; Rotman, 1961; Zipser, 1963). Recently, Graven, Steers and Anfinsen, 1964) calculated the molecular weight of the dissociated bacterial enzyme to be between 50,000 and 60,000.

The mammalian β-galactosidases from liver differ not only in pH optima but also in molecular weights. To determine whether a di- or trimerization of the subunits of the enzyme is associated with a shift in pH optimum will require further investigation. The present work also indicates that differences in electrophoretic migration of closely related enzymes may be caused by differences in molecular weight.

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