JOURNAL OF BACTERIOLOGY, Oct., 1966 Vol. 92, No. 4
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Partial Purification and Physical Properties of *Bacillus megaterium* β-Galactosidase

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Received for publication 5 July 1966

Comparison of the physical properties of *Escherichia coli* and *Aeromonas formicans* β-galactosidases revealed similar structural features for these two enzymes (S. R. Rohlfing and I. P. Crawford, J. Bacteriol. **91**:1085, 1966). Certain immunological and catalytic differences were observed, indicating that the primary structure of the two proteins differs; amino acid analyses confirmed this. A report on the properties and inducibility of β-galactosidase in *Bacillus megaterium* (O. E. Landman, Biochim. Biophys. Acta **23**:558, 1957) prompted us to compare its structure with the foregoing examples. The *B. megaterium* enzyme fails to cross-react immunologically with β-galactosidase from *E. coli*; furthermore, glucose added to the reaction mixture stimulates *o* - nitrophenyl - β - D - galactoside (ONPG) hydrolysis, an effect not observed with *E. coli* enzyme.

B. megaterium WRU 370 (Western Reserve University Microbiology Department collection) was grown on R. Mangalo and J. T. Wachsman's basal medium (J. Bacteriol. **83**:27, 1962) supplemented with 0.2% lactose. Conventional fractionation techniques, including ion exchange chromatography and gel filtration, afforded 500-fold enrichment of enzyme specific activity, but sedimentation velocity patterns displayed two components of about equal magnitude. Starch gel electrophoresis showed one prominent enzymatically active band, migrating at almost twice the rate of purified *E. coli* β-galactosidase, and three faint, inactive bands.

A mixture of *B. megaterium* and *E. coli* β-galactosidases was passed through a Sephadex G-100 column. Figure 1 shows the complete separation achieved, with *E. coli* β-galactosidase preceding *B. megaterium* β-galactosidase in the elution pattern.

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The sedimentation coefficient of partially purified *B. megaterium* β-galactosidase was estimated by density gradient centrifugation (R. G. Martin and B. N. Ames, J. Biol. Chem. **236**:1372, 1961). With use of 25 μg of *E. coli* β-galactosidase as the standard (16.0*S*), three separate determinations yielded values of 8.0 ± 0.5*S*. The molecular weight of *B. megaterium* β-galactosidase was estimated by gel filtration on Sephadex G-200. The column, calibrated during studies on the B component of tryptophan synthetase (D. A. Wilson and I. P. Crawford, J. Biol. Chem. **240**:

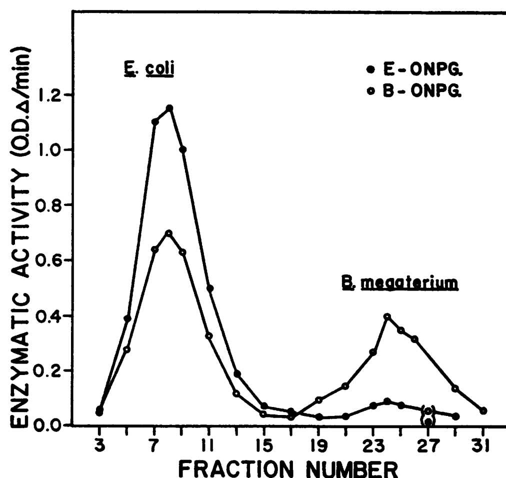

FIG. 1. *Separation of Escherichia coli and Bacillus megaterium β-galactosidases on Sephadex G-100. E-ONPG (1 mM ONPG in 5 mM sodium phosphate, pH 7.4) and B-ONPG (1 mM ONPG in 25 mM sodium phosphate, pH 7.4, plus 0.1 mM $MnCl_2$, 10 mM L-methionine, and 0.8 M D-glucose) are substrate solutions used for the differential assay of enzymatic activity. The activity ratio B-ONPG/E-ONPG for E. coli enzyme is 0.62; that for B. megaterium enzyme, 4.35. An enzyme mixture with an activity ratio of 1:00 was placed on the column (5 by 55 cm, eluted at 2 ml/cm²/hr with 25 mM sodium phosphate, pH 7.4, containing 0.1 mM $MnCl_2$ and 10 mM β-mercaptoethanol). Fractions 8, 17, and 24 had activity ratios of 0.62, 1.00, and 4.35, respectively. Points in parentheses (fraction 27) probably represent accidentally denatured enzyme.*

4801, 1965), yielded a linear relationship between the logarithm of the molecular weight of five protein standards (ranging from 29,500 to 151,000) and the ratio of their elution volumes to the void volume of the column (V/V_0). Duplicate determinations with *B. megaterium* β-galactosidase gave V/V_0 values of 1.25 and 1.24, providing an estimated molecular weight for the enzyme of 150,000.

These results indicate that *B. megaterium* β-galactosidase is much smaller than the β-galactosidase of either *E. coli* or *A. formicans*. Thus, in contrast to bacterial aldolases where molecular size is uniform (W. J. Rutter and W. E. Groves, p. 417, *in* C. A. Leone [ed.], Taxonomic biochemistry and serology, Ronald Press Co., New York, 1964), variations in the size of bacterial β-galactosidase may be pronounced, depending on the source of the enzymes compared. Although the taxonomic utility of these differences cannot yet be assessed, it seems clear that sufficient variation exists to justify characterization of the enzyme in other bacterial species.