PROPERTIES AND INDUCTION OF β-GALACTOSIDASE IN *BACILLUS MEGATERIUM**

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INTRODUCTION

For several years past, the enzyme β-galactosidase has been a focus of interest in enzyme biosynthesis studies[1-4] and, to some extent, in studies dealing with the mode of enzyme action[5]. This popularity is due not so much to the intrinsic importance of this enzyme but rather to the availability of a fast and accurate assay method. Further, β-galactosidase is generally inducible and is formed in abundant amounts in microbial cells in response to an array of inducing substances[3,4].

When WEIBULL's report[6] of protoplast formation in *Bacillus megaterium* offered a promising avenue of attack on the problem of enzyme biosynthesis in a subcellular system, the enzyme of choice in this laboratory[7] and elsewhere[8] was, of course, β-galactosidase.

In the following pages, the properties of *B. megaterium* β-galactosidase as well as its induction will be described. In the discussion section, *B. megaterium* data will be compared with the analogous ones from the model *Escherichia coli* system where by far the most extensive work has been done.

METHODS

Organism

In all of the work described in this paper, a lactose-utilizing variant of strain KM of *B. megaterium*[6] was employed. This strain is best maintained on lactose-minimal medium slants[9] containing 1 % lactose and 200 μg enzymic casein hydrolysate per ml, but fresh liquid 2 % peptone cultures can also be used as inocula. Older peptone cultures appear to offer a selective advantage to a rough, lactose-negative variant.

Cell free extracts

In most experiments, crude extracts were obtained by adding lysozyme at 200 μg per ml final concentration and methionine at 10^{-2} *M* to culture samples of known optical density. After ten minutes of incubation at 40° C, a clear extract is obtained which contains all of the activity of the cells.

Assay method

The assay method used is a modification of the procedure of AIZAWA[10] and LEDERBERG[11] in which the colorless substrate *o*-nitrophenyl-β-D-galactoside (ONPG) is hydrolyzed to form galactose and yellow *o*-nitrophenol.

The assay mixture, the origin of which will be described later, consists of the following ingredients: ONPG, 10^{-3} *M*; $MnCl_2$, 10^{-4} *M*; $NaPO_4$ buffer, pH 7.84, 10^{-1} *M*; glucose, $8 \cdot 10^{-1}$ *M*; and DL-methionine, 10^{-2} *M*.

In most experiments, 0.1 ml samples of enzyme are pipetted into matched 150 × 15 mm test

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tubes and lysed with 0.2 ml of lysozyme-methionine mixture for ten minutes at 40°C. Four ml of ONPG assay mixture at 40°C are then added and a "o" reading is taken in the Klett-Summerson colorimeter equipped with a No. 42 filter. After appropriate time intervals, subsequent readings are taken on the same tube. Activities are calculated from the resulting figures in terms of Klett units per ml of enzyme per minute and converted, when relevant, to specific activityunits in terms of mμmoles ONPG hydrolyzed per mg protein per minute. A linear relation between increment in O.D. and time obtains only if the following conditions are adhered to: (a) activity is not followed beyond the hydrolysis of 30% of the substrate; (b) dilution of enzyme is carried out with buffered 10^{-2} *M* methionine. The effect of dilution on enzyme activity in water and 10^{-2} *M* methionine is shown in Fig. 1.

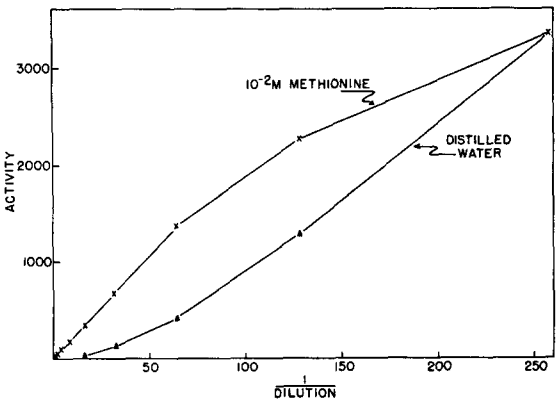

Fig. 1. Activity of β-galactosidase after dilution in 10^{-2} *M* methionine and distilled water.

Chromatography

For all chromatograms, except those involving sugar phosphates, ethyl acetate (100 vol.) – pyridine (50 vol.) – water (60 vol.) was employed as the solvent system[12]. Generally, Whatman No. 1 filter paper was used, and the chromatograms were developed in the descending manner at 29°C for 12 to 15 hours. After drying at room temperature, the papers were sprayed with a 2½% solution of aniline trichloroacetate in glacial acetic acid and heated for ten minutes at 100°C[13]. This procedure is capable of detecting 1 μg of reducing sugar.

After marking the reducing sugar spots, the papers were sprayed with a fresh 1% solution of $NaIO_4$. This agent was allowed to act for six minutes at room temperature. It was then followed by a spray of the following composition[14]: H_2O 47 ml; 95% EtOH 53 ml; benzidine 0.46 g; acetone 5 ml; 0.1 *N* HCl 5 ml.

This latter treatment exhibits both reducing and non-reducing sugars and glycosides as light spots on a dark blue background. It was used in combination with the aniline-trichloroacetate spray because the periodate-benzidine procedure is less sensitive.

For sugar phosphates, the solvents used were methanol (60 vol.) – concentrated NH_4OH (10 vol.) – H_2O (30 vol.); the paper was S and S No. 589. Ascending chromatograms were run at 4°C for 15 hours[15].

Inorganic phosphate and sugar phosphates were detected by the method of GANGULI[16].

Erlenmeyer-Klett-tube flasks

Special flasks were used to permit rapid observation of optical density during induction. A Pyrex tube 15 × 120 mm, which fits into the Klett-Summerson colorimeter, was sealed to the mouth of a 250 ml Erlenmeyer flask. A side arm located two-thirds from the bottom of the flask permitted introduction and withdrawal of material.

Growing cells for induction experiments

For the induction experiments cited in this paper, cells were grown in McQuillen minimal medium[9] plus trace elements, 200 μg/ml enzymic casein hydrolysate, 3 mg/ml Na citrate, and 0.1 *M* K_2HPO_4 buffer. The pH was adjusted to 7.0. When the culture had reached an O.D. of about 0.1, it was dispensed at the rate of 20 ml per flask into the Erlenmeyer-Klett-tube flasks containing appropriate amounts of inducer. These were then incubated on a rotary shaker at 29°C and O.D. measurements and samplings carried out at 30 to 60 minute intervals for a total of about 4 hours. In some experiments the samples were pipetted into large volumes of buffer, centrifuged out, and resuspended in fresh buffer in order to eliminate competitive inhibition by the inducers during assay. This inhibition is only significant, however, when inducers containing aromatic aglycon groups are used at concentrations in excess of 10^{-2} *M*. Washing can therefore be omitted in almost all experiments.

RESULTS

A. *Properties of* B. megaterium *β-galactosidase*

pH Optimum. In the standard assay mixture, the enzyme-ONPG reaction proceeds at a maximal rate between pH 7.3 and 8.0. Routine assays are carried out at pH

7.84 since the colored tautomer of *o*-nitrophenol is predominant at this pH. A pH-activity curve obtained with purified enzyme is shown in Fig. 2.

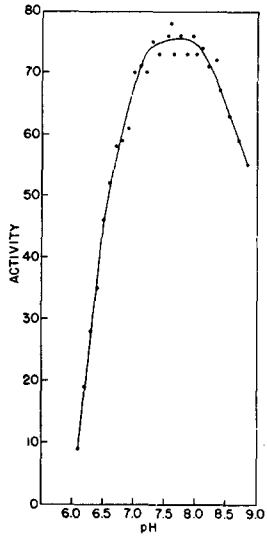

Fig. 2. Activity of β-galactosidase at various pH's.

Stability. In a controlled series of tests, crude lysozyme lysates retained full activity in 24 hours of storage at —20° C but lost 49 % of their activity in 12 days at the same temperature. If whole cells rather than lysates were stored, activity losses were similar. Storage at 0° C resulted in only slightly increased losses. On the other hand, a partly purified preparation was stored at —20° C for 60 days without any activity loss. The available data suggest that a proteinase is responsible for this disappearance of activity.

In addition to these irreversible losses in activity, *B. megaterium* β-galactosidase exhibits several reversibly inactive states. These can be recognized by the use of assay mixtures without methionine and assay mixtures containing 0.5 *M* phosphate buffer. Details concerning this reversible inactivation phenomenon will be described in a separate publication.

Thermal inactivation. The inactivation of β-galactosidase at elevated temperatures is strongly affected by the buffer content of the medium. Thus, a 5 minute exposure of variously buffered enzyme aliquots to 55° C resulted in 97 % inactivation for the distilled water sample, 42% inactivation for the one in 0.5 *M* K_2HPO_4 buffer, and no activity loss for a sample in 1.5 *M* K_2HPO_4.

Purification. In principle, purification has been accomplished by (a) securing selective release of β-galactosidase through partial cell lysis followed by (b) enzymic depolymerization or removal of nucleic acid contaminants with $MnSO_4$ or streptomycin, (c) $(NH_4)_2SO_4$ fractionation, and sometimes (d) starch column electrophoresis. Selective release of enzyme may be accomplished by suspending and shaking protoplasts in incompletely stabilizing media such as 0.25 *M* KH_2PO_4 buffer or 0.3 *M* sucrose. Samples are removed at intervals to determine the extent of the release. When complete, the preparation is centrifuged. The flow sheet from a typical purification is shown in Fig. 3.

Ion effects. To prepare enzyme for a study of the effects of ions on ONPG hydrolysis, a purified preparation of enzyme of specific activity 9700 was dialyzed for 8 hours against two changes of 0.6% versene plus 0.1 *M* methionine and for 6 hours against three changes of triple distilled water. The resulting preparation retained approximately one-fourth of its original activity. When 0.1 *M* methionine was used during the second dialysis, about one-half of the activity remained. Table I shows the effect of alkali cations and Mn^{++} as well as some anions on the rate of ONPG hydrolysis by the water-dialyzed preparation.

It is evident that Na^+ ion stimulates far more efficiently than K^+ ion, which in turn is superior to NH_4^+. Li^+ and Cs^+ are comparable to NH_4 in activity. Among the anions, arsenate is slightly better than phosphate, which in turn is superior to pyrophosphate and tris(hydroxymethyl)aminomethane buffer. Although an appreciable amount of activity is observed in the absence of added Mn^{++} ion, activity in its presence is approximately tripled. Mn^{++} also stimulates K^+ and NH_4^+-activated enzyme. It is not replaced by Mg^{++}.

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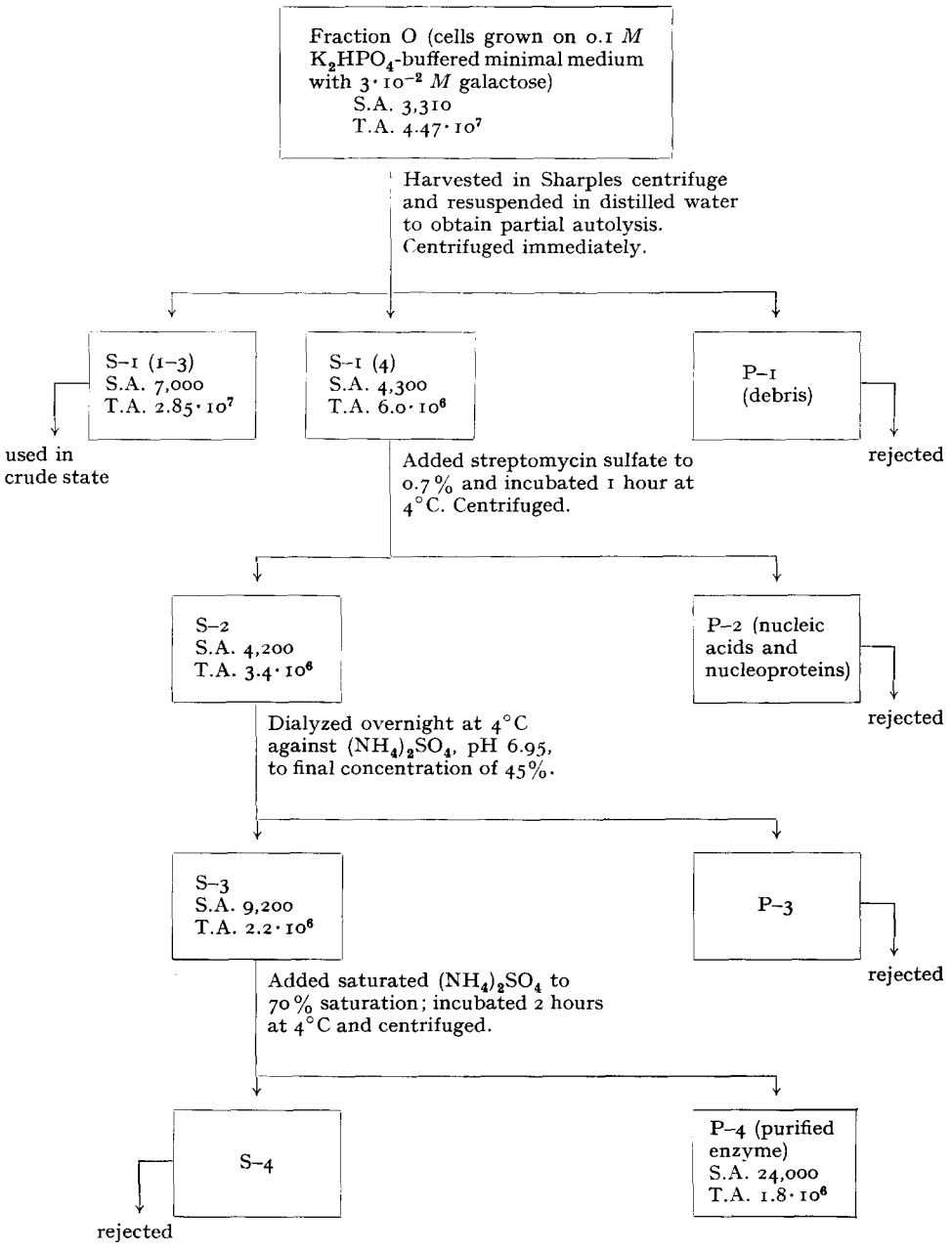

S = Supernatant Fraction;
S.A. = Specific Activity;
P= Pellet Fraction;
T.A. = Total Activity.

Fig. 3. Flow sheet for the purification of *B. megaterium* β-galactosidase.

TABLE I

ION ACTIVATION OF β-GALACTOSIDASE

Reagent	$MnCl_2$	*Glucose*	Na_2HPO_4	K_2HPO_4	$(NH_4)_2HPO_4$	Na_2HAsO_4	$Na_4P_2O_7$	*"Tris"*	*Activity*
pH			*7.84*	*7.84*	*7.8*	*7.74*	*7.84*	*7.8*	
Final conc.	10^{-4} *M*	*0.8 M*	*0.1 M*	*0.1 M*	*0.1 M*	*0.05 M*	*0.05 M*	*0.05 M*	
1*									0
2	+								0
3			+						155
4				+					17
5					+				1
6	+		+						410
7	+	+	+						1150
8	+	+				+			1490
9	+	+					+		155→0
10	+	+						+	927→646
11	+	+							0

* All tubes contain 10^{-3} *M* ONPG.

The most unusual of the effects exhibited in Table I is the stimulation by 0.8 *M* glucose. This stimulation is not specific to glucose but is also observed to a lesser extent with 0.8 *M* fructose, 0.2 *M* sucrose, and 0.2 *M* maltose. Melibiose and raffinose are inactive. This lack of specificity is only one of several features of the glucose effect which suggest that the stimulation can not be attributed to an acceptor or catalytic role of this sugar. Thus (a) substantial activity is observed in the complete absence of glucose; (b) the optimal glucose concentration is 800-fold that of the substrate; (c) in the absence of methionine, glucose stimulates Na^+-activated enzyme but inactivates K^+-activated enzyme. In the presence of methionine, glucose stimulates with both cations.

The stabilizing effect of DL-methionine is not confined to this amino acid and is thus also nonspecific. Both alanine and 1–10 mg/ml of crystalline bovine albumen can replace methionine.

Products and specificity range of β-galactosidase action. The failure of orthophosphate to reverse enzyme inactivation by pyrophosphate and "tris" buffers, coupled with the high activity of purified dialyzed enzyme in "tris"-NaCl buffer (see Table I), strongly suggests that *B. megaterium* β-galactosidase does not require phosphate for its activity. This tentative conclusion was confirmed by a paper-chromatographic analysis of the products of β-galactosidase action.

By use of the procedures outlined under METHODS, it was found that glucose, galactose, and an unidentified reducing saccharide—presumably an oligosaccharide—are the products of *B. megaterium* β-galactosidase action on lactose. The relative quantities of these products varied from one experiment to the next, however, and on some chromatograms galactose and the slow-moving reducing sugar spot were altogether absent. This variability led to renewed efforts to find evidence for the production or utilization of phosphorylated sugars. No such intermediates were found on any of the chromatograms, however, and tests for utilization of galactose-1-phosphate in the purified enzyme system were entirely negative. In the course of these paper-chromatography experiments, it was observed that the addition of phosphate, arsenate, and Mn^{++} ions greatly accelerated the cleavage of lactose, just as these substances stimu-

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TABLE II

PROPERTIES AND INDUCTION OF *B. megaterium* β-GALACTOSIDASE IN RELATION TO VARIOUS SUGARS AND GLYCOSIDES

Substances	*Split by purified enzyme*	*Used as growth substrate*	*Enzyme complexing properties* K_M *or* K_I	*Induces*	*Lowest conc. giving maximal induction*	*Maximal E–F rate*	*Approximate inducer conc. yielding ½ maximal rates*
D-Galactose	o	+	K_I $1.6 \cdot 10^{-2}$	+	$3 \cdot 10^{-2}$ M+	4.57(+)	$3 \cdot 10^{-3} - 10^{-2}$
Lactose	+	+	K_I $1.8 \cdot 10^{-3}$	+	10^{-2} M	2.00	$3 \cdot 10^{-3}$
Methyl-β-D-galactoside	+	+	K_I $3 \cdot 10^{-2}$	+	$3 \cdot 10^{-3}$ M	0.87	$10^{-4} - 3 \cdot 10^{-4}$
Thiomethyl-β-D-galactoside	o	o	K_I $8.1 \cdot 10^{-4}$	+	$3 \cdot 10^{-3}$ M	0.93	$10^{-4} - 3 \cdot 10^{-4}$
Phenyl-β-D-galactoside	+	+	K_I $2.4 \cdot 10^{-4}$	+	$3 \cdot 10^{-3}$ M	1.49	—
Thiophenyl-β-D-galactoside	o	o	K_I $3.2 \cdot 10^{-4}$	+	$3 \cdot 10^{-3}$ M	1.85	$3 \cdot 10^{-4}$
L-Arabinose	o	+	*	+	$3 \cdot 10^{-3}$ M	1.70	10^{-3}
ONPG	+	**	K_M $1.6 \cdot 10^{-4}$	+	—	—	—
n-Butyl-β-D-galactoside	+	+	Inhibitor	+	—	—	—
p-Amino-thiophenyl-β-D-galactoside	o	o	Inhibitor	+	10^{-2} M	4.8(+)	$10^{-4} - 3 \cdot 10^{-4}$
Melibiose	o	+	*	+	$3 \cdot 10^{-2}$ M	—	—
Methyl-β-L-arabinoside	o	o	*	o	—	—	—
Galacturonic acid	o	o	—	o	—	—	—
Xylose	o		—	o	—	—	—

* No inhibition observed at 1000 × ONPG concentration.
** Growth inhibited due to release of toxic ONP.

late ONPG hydrolysis. By contrast, K^+ ion appeared to be as effective as Na^+ ion in stimulating lactose cleavage.

The capacity of *B. megaterium* β-galactosidase to hydrolyze a series of β-galactosides, β-thio-galactosides, a β-arabinoside, and an α-galactoside was examined in another set of experiments. Results are noted in Table II. It is seen that in addition to lactose and ONPG the enzyme hydrolyzes methyl-, butyl-, and phenyl-β-D-galactosides. On the other hand, no evidence of galactose or arabinose liberation was obtained when high concentrations of enzyme were incubated overnight with thiomethyl- and thiophenyl-β-D-galactosides or with methyl-β-L-arabinoside. Further, the apparent hydrolysis of the α-galactoside melibiose by a purified enzyme preparation could be traced to a contaminating α-galactosidase. Only β-galactosidase samples which had been purified by starch column electrophoresis were free from this contamination.

Affinity relations. When ONPG is used as the enzyme substrate, a typical Michaelis-Menten relationship is obtained with concentrations ranging up to $2 \cdot 10^{-3}$ M. The Michaelis constant is estimated from the Lineweaver-Burk plot (Fig. 4) at $1.6 \cdot 10^{-4}$ M. With ONPG concentrations beyond $4 \cdot 10^{-3}$ M, a marked inhibition is observed; thus, at $8 \cdot 10^{-3}$ M ONPG, the hydrolysis rate is only one-half of maximal.

The affinities of some of the other galactosides for β-galactosidase were measured in a series of tests in which the inhibition of ONPG hydrolysis by a fixed concentration of inhibitor was estimated at various ONPG concentrations. From such data (Fig. 4) a K_I (competitive inhibition) was calculated[17] for several relevant substances and is given in Table II.

Crypticity. In *B. megaterium*—just as in *E. coli*[11] and yeast[2]—the β-galactosidase activity of lysates is much higher than that of the corresponding whole cell preparations. The crypticity ratio, $\frac{\text{lysate activity}}{\text{intact cell activity}}$, is variable and increases with the level

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of adaptation[18]. It is higher in organisms adapted at a high pH. Most important, it was found that the crypticity of a given cell preparation could be drastically modified by a few hours' incubation in media of different ionic strength and composition. For example, a cell suspension of an initial crypticity of 10 gave values of 2.3 and 18.6 after 12 hours' incubation in 0.05 and 0.5 M K_2HPO_4 buffer, respectively. All preparations gave the same activity upon lysis. Similar behavior is observed in protoplasts.

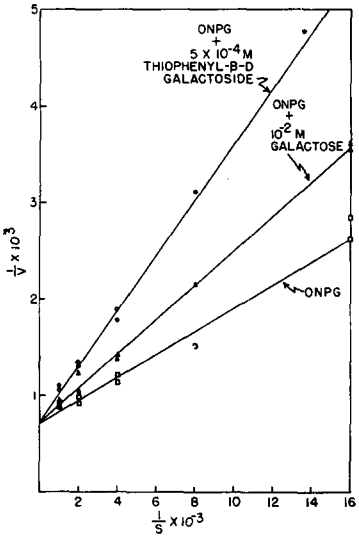

Fig. 4. Lineweaver-Burk plot for the determination of the Michaelis constants for ONPG and the competitive inhibition constants for galactose and thiophenyl galactoside.

B. *Induction of* B. megaterium *β-galactosidase*

In order to carry out a meaningful comparison between the inducer properties of various substances, it is important to provide a medium which allows for maximal expression of inducer activity. In a survey designed to discover such a medium, it was found that the basal salts, the concentration of amino acids, and especially the nature and concentration of the energy source all affect the induction levels attainable with a given concentration of inducer. Further, since some of the inducers are utilized in preference to the energy sources tested, while others are not used at all, the interrelationships between inducer and energy source and inducer and amino acid supplement are also dependent on the particular inducer used.

Basal medium. Without attempting to explore these complexities in detail, an induction medium was sought which would allow maximum enzyme development with the inducer thiomethyl-galactoside. This substance is not used for growth by *B. megaterium* and is therefore suitable to test the adequacy of various substances as "neutral energy sources" in induction experiments. In a survey of several likely compounds at a 1 mg/ml level, it was found that citrate and succinate did not interfere with induction when compared to a "control" without exogenous energy source. Acetate and glycerol interfered only slightly while fructose and especially glucose inhibited severely. In the same experiment, it was also found that an increase in the amino acid concentration from 200 μg/ml to 1000 μg/ml and to 10,000 μg/ml resulted in a 14% and a 28% drop in the specific activity level.

Following a series of such experiments, it was decided to use 3 mg Na citrate per ml as the carbon and energy source and 200 μg enzyme-hydrolyzed casein per ml as the source of amino acids. McQUILLEN's medium[9] plus trace elements serves as the basal medium.

Nonutilizable inducers. For many experiments in the field of induced enzyme biosynthesis, a nonmetabolizable inducer is an invaluable tool[19]. In *B. megaterium* three nonutilizable inducers are available: thiomethyl-β-D-galactoside, thiophenyl-β-D-galactoside, and *p*-aminothiophenyl-β-D-galactoside. At concentrations up to $3 \cdot 10^{-2}$ M, the thio-galactosides do not affect the growth rate of *B. megaterium* on citrate minimal medium, and no increase in final optical density is observed beyond that of the citrate control when the citrate concentration is limiting. These observations

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have been confirmed for the thiomethyl and thiophenyl derivatives using a heavy β-galactoside-adapted cell suspension as the inoculum. They are consistent with the observed inability of β-galactosidase to split the thio-galactoside linkage.

Optimum pH for adaptation. Using $3 \cdot 10^{-2}$ *M* thiomethyl-galactoside as the inducer in the standard medium buffered with 0.1 *M* K_2HPO_4 buffer, a determination of the optimum pH for adaptation was made. The range from pH 7.8 to pH 5.6 was covered in steps of 0.2 to 0.3 pH units. At the end of a 4 3/4 hour incubation period, the pH in all tubes had risen by 0.20 to 0.52 units. The course of enzyme formation at representative pH's is exhibited in Fig. 5. At intermediate pH values, the curves assume intermediate shapes and slopes. The data in Fig. 5 are plotted in terms of activity per μg protein[19,20] so that differences in the rate of enzyme formation due to alterations in growth rate are eliminated. In the pH range of 7.3 to 6.5, enzyme formation appears to follow a linear course starting with the moment of inducer addition[19]. The kinetics of enzyme appearance in the low pH range are evidently more complicated, but no effort was made to investigate this in detail.

Enzyme formation rates with various inducers. Using standard induction medium buffered with 0.1 *M* K_2PHO_4 buffer at pH 7.0, experiments have been performed to determine enzyme formation rates for eight different inducers of β-galactosidase. In every case except, possibly, that of phenyl-β-D-galactoside, the plot of increment in enzyme versus protein increase follows a linear course at optimal inducer concentration. At inducer concentrations beyond the optimal, this "differential rate of synthesis"[19] remains the same. Actually, it is somewhat surprising to obtain a steady induction rate with lactose and with methyl-galactoside, since both are hydrolyzed to yield the superior inducer, galactose. On the other hand, the apparent nonlinearity of induction with phenyl-β-D-galactoside can perhaps be attributed to secondary induction by liberated galactose.

The interpretation of induction kinetics at inducer concentrations below saturation

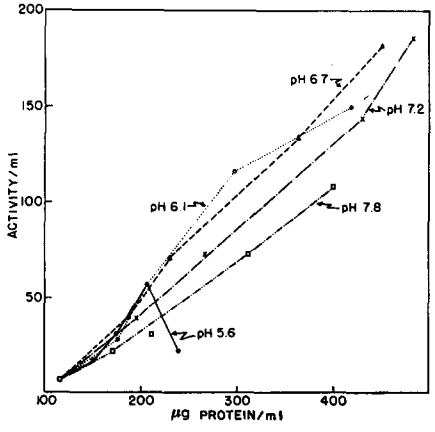

Fig. 5. The kinetics of enzyme appearance in cells induced by thiomethyl-galactoside at various pH's. Enzyme increase is plotted as a function of protein increase.

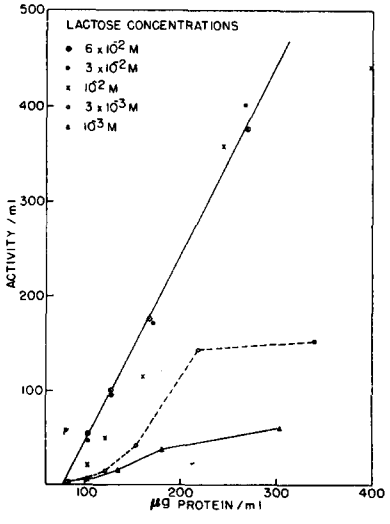

Fig. 6. The kinetics of enzyme appearance in cells induced by various concentrations of the utilizable inducer lactose.

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is less reliable, especially for utilizable inducers which are present in continuously decreasing amounts under the conditions of the experiments. Nevertheless, the rate of enzyme appearance in the earlier part of the inductions should give an approximation of the affinity of the inducer or the stimulated system. As an illustration of induction at inducer levels below saturation, the curves for lactose induction at various concentrations are exhibited in Fig. 6. With nonutilizable inducers, estimates of stimulatory activity at low concentrations are more reliable, but, as is evident from Fig. 7, the course of thiophenyl-galactoside-induced enzyme appearance is likewise not linear at concentrations below saturation.

Estimates for differential synthesis rates for varying concentrations of all of the eight inducers tested are plotted semilogarithmically against inducer concentrations in Fig. 8. This figure, then, gives a rough indication of the affinity of the various inducers in stimulating induction and a fairly good measure of the differential rate of synthesis for each inducer at saturation. Numerical data for the principal features of Fig. 8 are given in Table II.

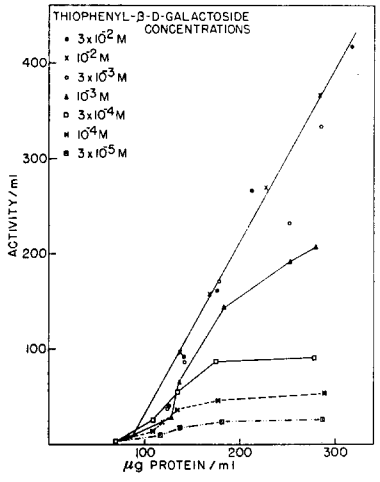

Fig. 7. The kinetics of enzyme appearance in cells induced by various concentrations of the nonutilizable inducer thiophenyl-galactoside.

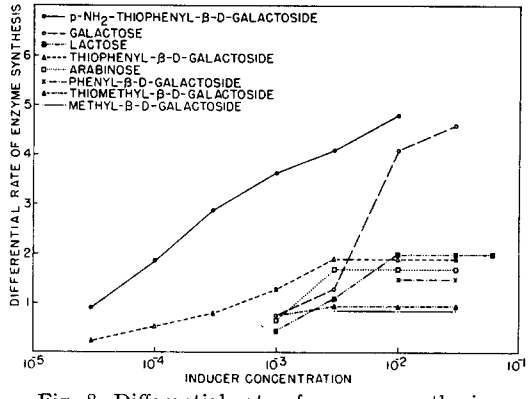

Fig. 8. Differential rate of enzyme synthesis as a function of inducer concentration. Inducer concentration is plotted on a logarithmic scale.

DISCUSSION

One of the principal matters of interest in a description of *B. megaterium* β-galactosidase lies in the comparison of this enzyme with the homologous one from *E. coli*. Data from the *coli* β-galactosidase system have constituted the major support for many generalizations concerning induction[4], and the discovery of analogous behavior in another bacterial family would therefore serve as an important corroboration for these generalizations.

From the available date, it appears that the catalytic properties of *B. megaterium* β-galactosidase are fairly similar to those of the *E. coli* enzyme. Both are activated primarily by Na^+ ion when acting on ONPG, and they can be further stimulated by the addition of Mn^{++} ion[21]. Further, as shown in Table III, the same substrates are attacked by the two enzymes, and the affinities of both substrates and competitive inhibitors for the two enzymes are of the same order of magnitude[22,23]. In addition, it may be noted that both enzymes have pH optima between pH 7 and 8; both are

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TABLE III

COMPARISON OF *E. coli* AND *B. megaterium* β-GALACTOSIDASES

Substances	*B. megaterium enzyme*		*E. coli enzyme*		*Relative inducer activity at saturation (Lactose = 100)*	
	Substrate	K_M *or* K_I	*Substrate*	K_M *or* K_I	*B. megaterium*	*E. coli*
Lactose	+	$1.8 \cdot 10^{-3}$	+	$1.1 \cdot 10^{-3}$	100	100
Galactose	o	$1.6 \cdot 10^{-2}$	o	$9.4 \cdot 10^{-3}$	288	17
Methyl-β-D-galactoside	+	$3 \cdot 10^{-2}$	+	$6.9 \cdot 10^{-3}$	43	112
n-Butyl-β-D-galactoside	+	—	+	$6.9 \cdot 10^{-4}$	+	112
Phenyl-β-D-galactoside	+	$2.4 \cdot 10^{-4}$	+	$7.3 \cdot 10^{-4}$	74	22
o-Nitrophenyl-β-D-galactoside	+	$1.6 \cdot 10^{-4}$	+	$1.8 \cdot 10^{-4}$	+	42
Thiophenyl-β-D-galactoside	o	$3.2 \cdot 10^{-4}$	o	$2.6 \cdot 10^{-4}$	92	o
Thiomethyl-β-D-galactoside	o	$8.1 \cdot 10^{-4}$	o	—	46	>100
L-Arabinose	o	o	o	o	85	o
Melibiose	o	o	o	2.10^{-2}	+	96

inhibited by "tris" buffer and both can act as trans-galactosidases under appropriate conditions[24].

The *E. coli* and *B. megaterium* enzymes do differ with respect to their stability and in their serological reactions. While the *E. coli* enzyme is extremely stable in both crude lysates and in a highly purified state, *B. megaterium* enzyme is destroyed very rapidly on dilution in water (see Fig. 1) and at an appreciable rate in lysozyme-lysates or whole cells, even at —20° C. Both enzymes are precipitated but not inactivated by their homologous rabbit antisera. *E. coli* anti-β-galactosidase did not precipitate any *B. megaterium* enzyme (M. COHN, personal communication, 1956).

A comparison of the induction patterns in *E. coli* and in *B. megaterium* reveals some parallelisms and some divergencies. Superficially at least, the kinetics of enzyme formation at inducer saturation are linear in both organisms when enzyme increase is plotted as a function of protein increase. Further, many of the same substances, both utilizable and "nonutilizable", are active inducers in both bacteria. On the other hand, there are decided differences; L-arabinose and thiophenyl-β-D-galactoside are both good inducers in *B. megaterium* and, respectively, inactive or inhibitory in *E. coli*; galactose and phenyl-β-D-galactoside are good inducers in *B. megaterium* but poor inducers in the wild *E. coli* strain (see Table III).

The results obtained with the *B. megaterium* β-galactosidase system support several of the conclusions which were derived from earlier experiments with *E. coli*[23] and with yeast[25]: (a) The existence of nonutilizable inducers rules out the hypothesis that enzyme induction is linked to utilization of the enzyme substrate. (b) The lack of correlation between enzyme-inducer affinity on the one hand and induction efficiency on the other (Table II) precludes any hypothesis of inducer action which is based primarily on a combination between inducer and enzyme. This lack of correlation is underlined by the comparisons between induction in *E. coli* and *B. megaterium* made in Table III. If an inducer-enzyme combination played a significant role, the catalytically similar *coli* and *megaterium* enzymes would be expected to entrain similar induction patterns. This is obviously not the case.

These considerations lead to a hypothesis of inducer action which postulates a combination of inducer with enzyme-forming system rather than with enzyme. Thus,

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the inducer is assigned a catalytic role in enzyme formation. This "catalytic" hypothesis has found compelling support in the finding of POLLOCK AND TORRIANI[26] that 20 molecules of *B. cereus* penicillinase may be formed as the result of the fixation of a single molecule of penicillin.

Actually, even the catalytic hypothesis of enzyme formation envisages a transient combination between enzyme in the process of synthesis and inducer fixed to the enzyme-forming site. If we disregard the implausible possibility that the inducer does not partake in the formation of the catalytic site of the enzyme molecule, we are still left with the task of explaining the lack of correlation between inducer activity and enzyme-inducer affinity. At present, a clear explanation of this lack of correlation is not available, but there are several known complications which might well be responsible for the observed complexities: (a) Several pieces of evidence from the *E. coli* system suggest that at least some of the inducers must be metabolized before they can act inside the cell. The nature and concentration of the external inducer thus may bear little relation to the intracellular situation. (b) In recent months COHEN AND RICKENBERG[27] have discovered a special inducible transport system for inducers of β-galactosidase in *E. coli*. Differences in "permeability" to different inducers thus certainly play a role in determining inducer activity. (c) All correlations between enzyme-inducer affinity and induction activity are based on the assumption that the cell-free enzyme on which measurements are made is identical with the intracellular enzyme. This assumption may well be incorrect. Certainly, many of the observations made on the crypticity phenomenon in *B. megaterium* (LANDMAN, unpublished observations, 1955), *E. coli*, and yeast[28] are strongly suggestive of a structural alteration in the enzyme molecule upon release from the cell. In view of these uncertainties, engendered by the use of whole-cell preparations, the decisive explanations of the sequence of events in inducer action will probably emerge from a study of subcellular systems. Such studies are now in progress[29].

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SUMMARY

The β-D-galactosidase of *B. megaterium* hydrolyzes β-D-galactosides to galactose and the aglycon alcohol. It is activated by Na^+ and Mn^{++} ions and 0.8 *M* glucose and is stabilized by 0.01 *M* methionine. The pH optimum is near pH 7.7. Purified β-galactosidase splits methyl-, *n*-butyl-, phenyl-, *o*-nitrophenyl-, and glycosyl-β-D-galactosides but does not attack the β-D-thiogalactosides, methyl-β-D-arabinoside, or glycosyl-α-D-galactoside. The activation characteristics and substrate specificity of *B. megaterium* β-glactosidase are similar to those of the homologus *E. coli* enzyme. Further, the two enzymes show a close correspondence in the numerical values of their affinity constants for the various substrates and inhibitors.

By contrast, β-galactosidase induction in *B. megaterium* exhibits a pattern distinct from that of *E. coli*. L-Arabinose and thiophenyl-β-D-galactoside are active inducers in *B. megaterium* and inactive or inhibitory in *E. coli*. In addition, there is a complete lack of parallelism in the quantitative inducer efficiency of the various galactose derivatives in the two organisms. In both bacteria, the kinetics of enzyme appearance is linear at inducer saturation.

The implications of the foregoing results relevant to theories of enzyme induction are discussed.

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THE CONVERSION OF FIBRINOGEN TO FIBRIN

XX. TRANSVERSE WAVE PROPAGATION AND NON-NEWTONIAN FLOW IN SOLUTIONS OF INTERMEDIATE FIBRINOGEN POLYMERS

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INTRODUCTION

The intermediate polymers of fibrinogen formed in the course of clotting with thrombin[1], which can be stabilized against further aggregation by certain inhibitors[1–3] or at high pH[4,5], are believed to be rather stiff long rods of uniform width and varying length. Their cross-section area is twice that of the fibrinogen monomer[6], corresponding to a diameter of the order of 50 A, while their length, though variable[4,7], is at least of the order of 5000 A.

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