A Locus Determining \( \beta \)-Galactosidase Activity in the Mouse*

James Felton, Miriam Meisler, and Kenneth Paigen

From the Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14208

SUMMARY

A group of inbred mouse strains, typified by C3H/HeJ, have twice as much \( \beta \)-galactosidase activity in their tissues as mice of a second group of strains represented by DBA/2J. In all organs tested, the activity in C3H/HeJ is expressed throughout development. The difference in activity is determined by a locus designated \( \beta \)-galactosidase (Bgs) on chromosome 9. The alleles present in C3H/HeJ (Bgs) and in DBA/LiHa (Bgs') show additive inheritance.

Studies of pH optima, heat lability, intracellular location, and molecular weight of the \( \beta \) galactosidase activity in crude homogenates suggest that only a single enzyme component is present. Partially purified enzyme preparations from C3H/HeJ and DBA/2J were indistinguishable with respect to heat lability, \( K_m \) for \( p \)-nitrophenylgalactoside, and molecular weight. The presence of a single enzyme component, the absence of detectable structural differences, and the additive inheritance of Bgs alleles suggest that this locus may be either a structural gene duplication or a regulatory site controlling the transcription or translation of a closely linked \( \beta \)-galactosidase structural gene.

MATERIALS AND METHODS

Animals

Mice for the strain survey and for developmental studies were obtained from the Research Stocks and Production Department of the Jackson Laboratory, Bar Harbor, Maine. For genetic crosses, mice of strains DBA/LiHa (previously known as DBA/2Ha (1)) and C3H/HeHa were kindly provided by Dr. T. S. Haushka, Roswell Park Memorial Institute. Mice were maintained on a 12-hour light, 12-hour dark schedule, and fed ad libitum on Rockland Mouse Breeder Diet (Teklab, Inc., Mont-Imou, Ill.) containing 17% minimum crude protein, 10% minimum crude fat, and 26% maximum crude fiber.

Tissue Homogenates

Freshly dissected tissues were weighed and homogenized in 0.25 M sucrose-0.02 M imidazole buffer, pH 7.0, (8 to 10%, w/v) with a Polytron homogenizer (Brinkmann) for 1 min. Homogenates stored at \(-20^\circ\) retained their \( \beta \)-galactosidase activity for 2 years.

Enzyme Assay

In our standard assay, reaction mixtures contained: 0.9 ml of 0.1 M citrate buffer, pH 3.5; 0.05 ml of 0.1 M \( p \)-nitrophenyl-\( \beta \)-galactoside (Pierce); and 0.06 ml of tissue homogenates. Reactions were started by addition of substrate, incubated for 30 to 60 min, and stopped by cooling in an ice bath followed by rapid addition of 0.2 ml of 30% trichloroacetic acid. After centrifugation for 10 min to remove precipitated protein, the supernatant solutions were transferred to tubes containing 0.2 ml of 5.0 M 2-amino-2-methyl-1:3-propanediol (Aldrich). The absorbance of released \( p \)-nitrophenol was measured in a Zeiss spectrophotometer at 415 nm (\( E_{1%} = 14,000\)).

The reaction rate was proportional to enzyme concentration and to time of incubation for more than 3 hours. Enzyme activity is expressed as micromoles of \( p \)-nitrophenol produced per hour.

Automated Assay of \( \beta \)-Galactosidase

For analysis of the large numbers of animals generated in genetic crosses, an auto-analyzer was designed which measures hydrolysis of the fluorogenic substrate 4-methylumbelliferyl-\( \beta \)-
The activity of murine \( \beta \)-galactosidase towards the 4-methylumbelliferyl derivative is approximately one-third of the activity toward the \( \beta \)-naphthyl compound. The 4-methylumbelliferyl\( \beta \)-galactosidase assay was used exclusively to analyze the segregation of \( \beta \)-galactosidase activity in genetic crosses.

Presentation of Data

In graphing frequency distributions, we have indicated the number of animals whose activity falls into each class interval. The class intervals employed follow a logarithmic scale in which the upper limit of each interval is 1.06 times the value of the lower limit (0.025 log unit). We do so because the populations we have studied have relatively constant coefficients of variation (standard deviation/mean), but quite different means. The use of a logarithmic scale is advantageous in comparing such populations since the coefficient of variation bears a constant relationship to the width of a class interval; as a result, the spread of each frequency distribution becomes independent of the absolute values of its mean. This is especially obvious in examining genetic crosses whose progeny segregate into distinct activity classes. The logarithmic scale avoids the artificial compression of the lower activity group, or the scatter of the higher group, that attends the use of a linear scale.

Protein

This was determined by the biuret method (6) with bovine serum albumin as standard. When necessary, turbid solutions were clarified after color development was complete by extraction with an equal volume of ether.

Enzyme Purification

Thirty-fold purification of mouse liver \( \beta \)-galactosidase was obtained by modification of a procedure for human liver \( \beta \)-galactosidase (7). All steps were carried out at 4°C.

Step 1—Livers from 12 to 18 mice were pooled and homogenized (20%, w/v) in 0.02 m phosphate buffer, pH 6.8, with a Polytron homogenizer for 2.0 min. Homogenates were frozen, thawed, and centrifuged for 30 min at 105,000 \( \times g \).

Step 2—The supernatant was diluted with an equal volume of buffer prior to the addition of ammonium sulfate (Mann ultrafiltration apparatus). The precipitate was collected by centrifugation at 105,000 \( \times g \) for 30 min. The clear supernatant was concentrated by ultrafiltration in an Amicon cell, with UM 10 filter. The concentrated enzyme preparation was stored when frozen for 6 months.

Heat Inactivation

In order to study the temperature dependence of \( \beta \)-galactosidase inactivation, a temperature gradient apparatus was constructed from a solid aluminum block, 20 \( \times \) 50 \( \times \) 8 cm, which was milled at each end to provide a channel for circulation of water. The block was insulated on all sides with \( \frac{1}{2} \) inch of polyurethane. A temperature gradient was maintained along the length of the block by circulation of water through each end at two different temperatures. Eight rows of wells for test tubes, 15 mm in diameter \( \times \) 60 mm in depth, were drilled along the length of the block. Tubes containing enzyme were incubated in these wells for 10 min. The temperature of each tube was measured with a Digitron digital thermometer.

Electrophoresis

Electrophoresis was performed in Tris-glycine buffer at pH 8.1 as previously described (5). Seven per cent polyacrylamide gels (5 \( \times \) 75 mm) were prerun for 15 min at 0° and 200 volts. Samples were subjected to electrophoresis for 15 min at 200 volts and then 90 min at 300 volts (about 2 ma per tube). After removal from running tubes, the pH of the gels was adjusted by incubation for 30 min in 0.1 m citrate, pH 3.5. \( \beta \)-Galactosidase activity was visualized with a staining solution (8) prepared as follows: 15 mg of 5-bromo-4-chloro-3-indolyl-\( \beta \)-galactopyranoside (Sigma) was dissolved in 0.5 ml of dimethylformamide, followed by the sequential addition of 24 ml of 0.1 m citrate buffer (pH 3.5), 0.3 ml of 0.85% NaCl, 6 mg of spermidine HCl, and 3.25 ml of a mixture containing equal volumes of 0.65 m potassium ferricyanide, and 0.05 m potassium ferrocyanide. The staining solution was stable for 2 days. After gels were stained at 57°C for 30 to 60 min, \( \beta \)-galactosidase appeared as a blue-green band.

Strain Variation

A set of 45 inbred mouse strains, chosen to provide as diverse a genetic pool as practicable, was surveyed for genetic variants of \( \beta \)-galactosidase. In order to detect structural variants, the electrophoretic mobility and thermal sensitivity of the enzyme were tested. To detect variants with altered intracellular location of enzyme, the relative amounts of soluble and particulate activity were assayed in liver. To detect regulatory and developmental variants, the quantitative levels of enzyme activity in a set of adult tissues were compared. No mutants in structure or subcellular location were detected among the set of 45 strains. However, we did find considerable variation in total enzyme activity among adult organs.

The strains could readily be divided into two groups on the basis of their brain enzyme activity. The members of one group show brain enzyme activities of 6 to 9 \( \mu \)moles per hour per g and those of the other group 13 to 16 \( \mu \)moles per hour per g. Brain activity was chosen as the distinguishing characteristic because there is very little individual variation in this activity, and because the distinction between the two groups is unambiguous (Fig 1). The values of brain \( \beta \)-galactosidase for these inbred strains is presented in Table 1.

We have chosen one strain from the high activity group, C3H/HeJ, and two strains from the low activity group, DBA/2J and C57BL/Ks, for further comparison. The value of \( \beta \)-galactosidase specific activity in adult tissues of C3H/HeJ mice is twice as high as the corresponding DBA/2J tissues (Table II).
Variation in specific activity of brain β-galactosidase among inbred strains of mice

Enzyme activity and protein concentration were determined on homogenates prepared from pooled tissues of three adult males from each strain.*

| Strain          | Micrometers per g | Strain          | Micrometers per g |
|-----------------|-------------------|-----------------|-------------------|
| AKR/J           | 6.6               | A/J             | 14.4              |
| AU/SsJ          | 7.0               | A/J             | 13.9              |
| BAN/Re          | 6.8               | BALB/c          | 13.3              |
| BDP/J           | 7.0               | CE/J            | 15.1              |
| BUB/BuJ         | 7.0               | C3H/HeJ         | 15.1              |
| CBA/J           | 7.7               | C57BL/6J        | 13.5              |
| CBA/CaJ         | 7.1               | C57BL/10J       | 14.8              |
| C57BL/KsJ       | 6.4               | C57Br/cdJ       | 15.1              |
| DBA/1J          | 5.7               | C57e/Ha         | 13.4              |
| DBA/2J          | 6.5               | C57L/J          | 14.4              |
| I/FnLn          | 8.9               | C57/J           | 14.9              |
| LG/J            | 8.8               | DE/J            | 16.0              |
| LP/J            | 8.8               | DW/J            | 16.3              |
| NZB/B1N         | 7.8               | FL/1Re          | 15.1              |
| P/J             | 7.4               | HRS/J           | 12.7              |
| PL/J            | 6.8               | MA/J            | 15.8              |
| RF/J            | 6.6               | MK/Re           | 14.9              |
| RI11/2J         | 6.8               | PH/Re           | 14.4              |
| SEC/1ReJ        | 7.7               | WB/Re           | 15.1              |
| SJL/J           | 5.6               | YBR/WiHa        | 15.7              |
| SM/J            | 7.7               |                 |                   |
| ST/J            | 8.3               |                 |                   |
| SWR/J           | 9.3               |                 |                   |
| WC/Re           | 7.5               |                 |                   |
| WH/Re           | 7.5               |                 |                   |
| WK/Re           | 8.0               |                 |                   |
| 129/J           | 8.1               |                 |                   |

* The data have been corrected for a 5% interstrain variation in protein concentration: the values are micromoles per hour per g wet weight divided by the factor:

\[
\frac{\text{protein concentration of specific strain}}{\text{average protein concentration of all strains}}
\]

Mixtures of samples from the 2 strains have the predicted additive activity, indicating that low molecular weight effectors are not responsible for the activity difference.

Development—The higher activity in C3H/HeJ relative to that in DBA/2J and C57BL/Ks does not appear to arise from a difference in the developmental pattern of the enzyme in these strains. Enzyme activities were measured in brain, liver, and heart of mice of each strain between gestational Day 14 and 60 days of age (Fig. 2A). The higher activity of C3H was maintained throughout this period, the activity in C3H being almost exactly twice that of the other strains in each tissue at all stages of development (Fig. 2B).

Enzyme Properties—The characteristics of the β-galactosidase activity present both in crude homogenates and in partially purified preparations from C3H/HeJ and DBA/2J livers were compared to determine whether the difference in activity reflected any difference in the properties of the enzyme. Several lines of evidence suggest that a single enzyme component is responsible for the β-galactosidase activity present in the tissue we have studied.

About 85% of the total β-galactosidase activity of liver was estimated to be in lysosomes from measurement of the relative amounts of soluble and particulate activity and the observation

---

Table I

| Strain          | Micrometers per g | Strain          | Micrometers per g |
|-----------------|-------------------|-----------------|-------------------|
| DBA/BJ          | 7.6 ± 0.1         | C3H/HeJ         | 16.4 ± 0.1        |
| C3H/HeJ         | 14.9 ± 0.5        | DBA/2J          | 2.1               |
| Heart           | 4.2 ± 0.2         | Kidney          | 2.0               |
| DBA/2J          | 71.0 ± 1.4        | C57BL/KsJ       | 1.7               |
| Spleen          | 40.0 ± 1.6        |                 | 2.1               |

Fig. 2. β-Galactosidase activity in developing mouse organs

Mice were obtained from the Production Department of the Jackson Laboratory and tissues were prepared as described (5). Birth was on Day 0. Liver, brain, and heart were studied in one high activity strain (C3H/HeJ (O-O)) and in two low activity strains (DBA/2J (O—O) and C57BL/Ks (D—D)). A, activities per g of tissue; B, relative activities. The data are expressed relative to the average value at each time point calculated from the DBA/2J, C57BL/Ks, and one-half of the C3H activities.
that nearly all of the particulate activity was released when the particulate fraction was exposed to hypotonic buffer (9). There was no difference between strains in this regard. The enzyme from the two strains had the same elution volume after gel filtration (see "Materials and Methods").

In every case the kinetics of heat denaturation of the activity present in crude homogenates was monophasic. There was no indication of more than one enzyme component. The sensitivity to thermal denaturation was measured over the range 37–49°C for partially purified enzyme (Fig. 3). From these data, a value of 39,000 cal per mole for the energy of activation of the denaturation reaction was calculated for both strains. The kinetics of denaturation was tested at 43°C and was first order with a rate constant of −0.07 min⁻¹ for both strains.

We have had considerable difficulty in carrying out acrylamide gel electrophoresis. Single bands with similar mobilities in all strains were observed at pH 8.1; however, the staining reaction requires high protein concentrations and the resolution was poor.

The enzyme present in both C3H/HeJ and DBA/2J liver has optimal activity at pH 3.5, and there was no difference in the pH activity curves of the two strains. Enzyme from both strains is completely inhibited by 10⁻³ M p-chloromercuribenzoate. The affinity of the enzyme for the substrate p-nitrophenyl-β-D-galactoside was determined (Fig. 4). A value of 5.2 × 10⁻⁴ M was obtained for the Kₘ of enzyme preparation from both strains.

Taken collectively, these results suggest that a single enzyme component is present in both strains, and that the difference in β-galactosidase activity between C3H and DBA mice appears to be controlled by a single locus with two alleles showing additive inheritance. The brain β-galactosidase activity of F₁ progeny of a cross between C3H and DBA showed intermediate levels of activity. Progeny of the backcross of the F₁ to the C3H parent segregated into the intermediate and high classes in approximately equal numbers (Fig. 5). Similarly, progeny of the backcross to the DBA parent segregated into the intermediate and low classes in approximately equal numbers (Fig. 5). The F₂ generation segregated into the expected three classes. The locus has been designated Bgs (β-galactosidase); the allele present in C3H/HeJ is Bgs¹ and that present in DBA/2J is Bgs².

**Table III**

| Coat color | Low (1.5–2.3 μmoles/hr/g) | Intermediate (3.4–4.5 μmoles/hr/g) | Total |
|------------|-----------------------------|----------------------------------|-------|
| Dilute     | 31                          | 10                               | 41    |
| Nondilute  | 7                           | 32                               | 39    |
| Total      | 38                          | 42                               | 80    |

Linkage tests to known loci (Table III), showed Bgs to be approximately 21.2 ± 4.6 centimorgans from the chromosome...
FIG. 5. Inheritance of β-galactosidase activity. Brain enzyme activity was determined for the parental strains C3H/HeHa and DBA/LiHa, and for the F1, F2, and both backcross generations. In all cases, reciprocal crosses were made, with no significant difference in the results. β-Galactosidase was determined by automated assay with the substrate 4-methylumbelliferyl-β-D-galactoside. All animals were between 9 and 12 weeks of age. Each filled circle represents one animal.

9 marker dilute. This agrees with the observations of Hakansson and Lundin.

Levels of β-galactosidase in liver and brain appear to be determined by the same locus. Animals segregating into the three brain activity classes, low, intermediate, and high, simultaneously segregate into the corresponding classes of liver activity (Fig. 6).

DISCUSSION

Inbred mouse strains show significant differences in levels of β-galactosidase; all of the strains examined could be classified as high β-galactosidase (specific activities between 13 and 16 μmoles per hour per g wet weight of brain tissue) or low β-galactosidase strains (6 to 9 μmoles per hour per g). In a representative high strain (C3H/HeJ), the β-galactosidase activity in all tissues at all stages of development is twice that of the low strains DBA/2J and C57BL/Ks. In genetic crosses between these strains, this quantitative difference segregated as a single Mendelian factor. Linkage tests demonstrated that the responsible locus, designated Bgs, is located on chromosome 9 in the mouse, approximately 21 centimorgans from the dilute locus. The enzyme level in heterozygotes was equal to the mean of the parental activities, i.e., the locus exhibits additive inheritance.

Two alternative models for the effect of the Bgs locus may be considered. First, the Bgs<sup>h</sup> allele (in C3H mice) may encode a structurally altered enzyme whose catalytic activity per molecule is twice as great as the enzyme encoded by the Bgs<sup>e</sup> allele (in DBA/2 mice). Alternatively, the effect of the Bgs<sup>h</sup> allele may be to increase the concentration of enzyme molecules in the tissues of C3H mice. The first model depends upon a difference in primary structure between the β-galactosidases present in the two strains; no differences in these parameters were observed. The probability that a structural change would be reflected in one of these parameters can be estimated. Empirically, 70% (39/55) of amino acid substitutions were found to alter the heat lability of bacterial β-galactosidase (11), and 67% (14/21) of variants in activity of glucose 6-phosphate dehydrogenase exhibited altered $K_m$ for substrate (12). Approximately 30% of base substitutions theoretically should produce a protein with altered net charge; however, we are not certain that a single charge difference would have been detected in our electrophoretic system. We feel that the absence of detectable differences in these three properties make it unlikely that the β-galactosidases of C3H/HeJ and DBA/2J mice differ in their primary structures and hence in their catalytic activity per molecule. However, definitive evidence on this point will require the purification of the enzyme and more detailed structural comparisons.

It appears more likely that the Bgs locus controls the number of enzyme molecules present in mouse tissues. Two models which account for the failure to detect any structural change and for the additive inheritance of the Bgs alleles are that Bgs<sup>h</sup> represents a duplication of the β-galactosidase structural gene, or that it represents an alternate form of a regulatory site controlling the efficiency of transcription or translation of a closely linked β-galactosidase structural gene.

In anticipation of further studies of the Bgs locus we wish to point out that all presently reasonable models for its action in-
dicate that Bgs is either identical with or closely linked to the structural gene for β-galactosidase.

In considering possible models of genetic regulation in mammals, it is of considerable interest that while β-galactosidase and β-glucuronidase are both present in lysosomes, and share a coordinated pattern of development (5), the genetic factors controlling their expression are not associated physically on the same chromosome; the Bgs locus is on chromosome 9, and the β-glucuronidase structural gene with its attendant regulatory sites is on chromosome 5 (1-4, 13).

Acknowledgments—We are grateful to Dr. Douglas Coleman of the Jackson Laboratory, Bar Harbor, Maine, for sponsoring our studies at the Jackson Laboratory during the summer months of 1970 to 1972. The strain survey and developmental studies were greatly facilitated by the cooperation of the Laboratory staff.

REFERENCES
1. Paigen, K. (1961) Exp. Cell Res. 25, 286
2. Ganschow, R., and Paigen, K. (1968) Genetics 69, 335
3. Swank, R. T., and Paigen, K. (1973) J. Mol. Biol. 77, 371
4. Paigen, K. (1961) Proc. Nat. Acad. Sci. U. S. A. 47, 1041
5. Meisler, M., and Paigen, K. (1972) Science 177, 894
6. Layne, E. (1961) Methods Enzymol. 3, 447
7. Meisler, M. (1972) Methods Enzymol. 28, 820
8. Yarborough, D., Meyer, O., Dannenberg, A., and Pearson, B. (1967) J. Reticuloendothelial Soc. 4, 390
9. Ganschow, R., and Paigen, K. (1967) Proc. Nat. Acad. Sci. U. S. A. 54, 938
10. Hofstee, B. H. J. (1952) Science 116, 329
11. Langridge, J. (1958) J. Bacteriol. 96, 1711
12. Yoshida, A. (1973) Science 170, 532
13. Sidman, R. L., and Green, M. C. (1965) J. Hered. 56, 23
A Locus Determining β-Galactosidase Activity in the Mouse
James Felton, Miriam Meisler and Kenneth Paigen

J. Biol. Chem. 1974, 249:3267-3272.

Access the most updated version of this article at http://www.jbc.org/content/249/10/3267

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/10/3267.full.html#ref-list-1