Genetic Determination of Amylase Synthesis in the Mouse*

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Extensive genetic variation in structure and rate of synthesis of pancreatic amylase has been identified among strains of mice. The relative rate of synthesis of amylase varies from 0.15 in strain YBR to 0.26 in strain C3H. The number of electrophoretic isozymes of pancreatic amylase varies between one and four. In each strain with multiple amylase isozymes, a characteristic quantitative distribution of protein among the isozymes is observed. Isozyme proportions are determined by the relative rates of synthesis of each component. Congenic lines with different amylase phenotypes have been established. Genetic analysis reveals the close linkage of cis-acting sites determining rate of synthesis and electrophoretic mobility of mouse pancreatic amylase.

Genetic variation affecting the rate of synthesis of specific proteins can be used to identify the chromosomical locations and modes of action of regulatory elements in the mammalian genome. Mouse amylases may be useful gene products for such studies. The enzymes exhibit extensive genetic variation (1–4), they are synthesized in abundance in specific differentiated tissues, and they can be readily purified for biochemical analysis.

In the mouse, pancreatic amylase and the homologous parotid enzyme are encoded by closely linked duplicated loci (2, 5). Both are monomeric enzymes of molecular weight approximately 56,000. The physical properties and developmental expression of mammalian amylases have recently been reviewed (6, 7).

Both quantitative genetic variation and qualitative (electrophoretic) variation affecting mouse pancreatic amylase have been described. Although the majority of inbred mouse stocks have only a single electrophoretic form of pancreatic amylase, double-banded phenotypes have been found among wild mice (2) and in a few inbred stocks (8, 9). Improved electrophoretic techniques have recently made possible the resolution of up to four components of pancreatic amylase from some of these double-banded animals (4, 10). Quantitative variation is evident within the multi-banded animals, as the different isoenzymes often occur in unequal amounts, resulting in electrophoretic patterns with "skew" proportions of the bands. Within a particular stock, the relative proportions of isozymes are constant, whereas marked differences are found among animals of different origin. Breeding experiments have demonstrated that this quantitative variation is under strict genetic control so that a given isozyme ratio is inherited as a Mendelian unit.

This study was initiated to determine whether the proportions of pancreatic amylase isozymes found in a given stock are a direct result of differences in production rate or whether other factors, such as differences in rate of degradation or secretion, may be involved. We have investigated the genetic determination of amylase expression in several mouse strains and will describe genetic variation affecting in vivo rates of amylase synthesis in the mouse pancreas.

MATERIALS AND METHODS

RESULTS

Two Pancreatic Amylase Isozymes in Strain YBR—Most strains of mice contain a single electrophoretic isozyme of pancreatic amylase (8–10). YBR is one of the exceptions, with two pancreatic amylase isozymes demonstrable by agar gel electrophoresis. The two isozymes, designated A, and B, can also be separated by electrophoresis at pH 8.1 in polyacrylamide gels (Fig. 1). There is a consistent difference in the concentration of the two isozymes in individuals of this strain. When pancreatic homogenates or purified amylase from YBR mice are electrophoresed and the gels stained for amylase activity, the B form predominates. However, the specific activities of the purified A and B isozymes do not differ. The relative amounts of protein in the two isozymes in pancreatic homogenates was quantitated by densitometry of protein-stained gels (Miniprint Fig. 1). Approximately 50% of the amylase protein is associated with the B isozyme; the ratio of B to A is 1.5:1.

To determine the basis for the unequal concentrations of these isozymes, we compared their relative rates of synthesis.

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1 Portions of this paper (including "Materials and Methods," Figs. 1S to 5S, and Tables 1S) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 80M-1048, cite author(s), and include a check or money order for $4.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 J. T. Nielsen, unpublished.

3 Strahler, J. H., Hewett-Emmett, D., and Meisler, M. (1980) Hereditas (abstr.).
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Amylase synthesis was analyzed by electrophoresis at pH 8.1 (Fig. 2). In heterozygotes from crosses of C3H and YBR mice, all three parental isozymes can be distinguished. We were therefore able to examine the rate of synthesis of each isozyme in heterozygotes.

If the two chromosomes in the heterozygotes contributed equally to amylase production, we would expect to find 50% of the heterozygote's amylase in the A2 isozyme, with the remainder divided between B1 and A1. However, when the protein concentration of the three isozymes was determined by densitometry, 70% of the amylase was of the A2 mobility, with 16% and 14% of the protein in isozymes B1 and A1, respectively. The relative rate of synthesis of the A2 isozyme was also twice as great as the sum of the A1 and B1 isozymes (Table 1). It is evident that the protein concentrations of the three isozymes are determined by their individual rates of synthesis. The isozyme from the strain with the 2-fold greater rate of amylase synthesis is also synthesized in 2-fold greater amounts.

**Table 1**

| Type of mice (n) | % of total amylase radioactivity | Relative rates of synthesis |
|------------------|---------------------------------|-----------------------------|
|                  | A1                              | A2 |
| YBR/Cv (8)       | 42 ± 3                          | 56 ± 3                      | 0.15 ± 0.02 (6) |
| C3H.Amy<sup>YBR</sup> (4) | 44 ± 1                          | 56 ± 1                      | 0.16 ± 0.02 (9) |
| C3H/As (6)       | 100                             | 0.24 ± 0.02                 |
| C3H/HeHa (7)     | 100                             | 0.26 ± 0.04                 |
| (C3H/As × YBR/Cv)F<sub>1</sub> (6) | 17 ± 2                          | 15 ± 2                      | 68 ± 3         |
| (C3H/As × C3H.Amy<sup>YBR</sup>)F<sub>1</sub> (10) | 17 ± 1                          | 18 ± 1                      | 65 ± 1         | 0.20 ± 0.01 |

"Materials and Methods." In strain YBR, amylase accounts for 15% of protein synthesized during a 15-min labeling period (Table I). This is unusually low for inbred mouse strains (10). Strain C3H/HeHa, which is typical, incorporates 26% of [3H]leucine into amylase during a similar labeling experiment (Table I). The results in the congenic line C3H.Amy<sup>YBR</sup> demonstrate that this quantitative trait is encoded by a site linked to the amylase structural gene region. The expression of this genetic trait is additive in heterozygotes (Table I).

The magnitude of the interstrain difference can be explained by a 2-fold elevation of the absolute rate of amylase synthesis in strain C3H, assuming that the rates of synthesis of the nonamylase proteins do not differ. This assumption seems justified especially in the congenic C3H.Amy<sup>YBR</sup> line, which differs from C3H only in the amylase gene region. (See Miniprint for discussion and additional data on the interstrain difference.)

A *cis*-Acting Site Determines the Interstrain Difference—

The isozyme pattern of C3H/HeHa mice is the common mouse pattern with a single pancreatic amylase isozyme, designated A<sub>2</sub>, which can be separated from the YBR isozymes by electrophoresis at pH 8.1 (Fig. 1). In heterozygotes from crosses of C3H and YBR mice, all three parental isozymes can be distinguished. We were therefore able to examine the rate of synthesis of each isozyme in heterozygotes.

If the two chromosomes in the heterozygotes contributed equally to amylase production, we would expect to find 50% of the heterozygote's amylase in the A<sub>2</sub> isozyme, with the remainder divided between B<sub>1</sub> and A<sub>1</sub>. However, when the protein concentration of the three isozymes was determined by densitometry, 70% of the amylase was of the A<sub>2</sub> mobility, with 16% and 14% of the protein in isozymes B<sub>1</sub> and A<sub>1</sub>, respectively. The relative rate of synthesis of the A<sub>2</sub> isozyme was also twice as great as the sum of the A<sub>1</sub> and B<sub>1</sub> isozymes (Table I). It is evident that the protein concentrations of the three isozymes are determined by their individual rates of synthesis. The isozyme from the strain with the 2-fold greater rate of amylase synthesis is also synthesized in 2-fold greater amounts.
quantities in the heterozygote, indicating that the trait is determined by a cis-acting site.

Additional Amylase Phenotypes—Several unique amylase phenotypes, differing in number and proportion of isozymes, were identified in a feral population. The characterization of congenic strains with these phenotypes is described in the accompanying Miniprint (Table I). All the investigated pancreatic amylase chromosome regions determine two types of information: the relative proportions of different isozymes and the overall rate of synthesis.

Expression of Isozymes in Newborn Mice—No major shift in the proportions of pancreatic amylase isozymes occur in the mouse after birth (see miniprint).

DISCUSSION

The observation that up to four pancreatic amylase isozymes are synthesized in homozygous mouse strains suggests that at least four structural genes may be active in these lines. Previous studies by heat inactivation and peptide mapping also revealed molecular heterogeneity of amylase within inbred strains (4). We have studied two kinds of quantitative elements encoded by cis-acting sites closely linked to the structural amylase genes.

Mice with one to four pancreatic amylase isozymes were identified by polyacrylamide gel electrophoresis of inbred stocks and feral populations. From some of the animals we have produced congenic lines on a C3H/As background. The lines with multiple amylase forms have distinctive proportions of isozymes in the pancreas. Within a line each amylase isozyme is synthesized in vivo at a unique rate which determines its contribution to the total amylase protein. In heterozygotes produced by crossing congenic lines with C3H, the parental ratios among isozymes are preserved. The three components of phenotypic variation encoded by the amylase gene region in congenic lines are summarized in Fig. 2.

Two alternative explanations for multiple amylase isozymes in homozygous and heterozygous mouse strains are possible: either they are encoded by duplicated copies of the amylase structural gene, or they result from processing of a single precursor nucleic acid or peptide. Two lines of evidence suggest that the isozymes do not result from post-translational modification. First, the isozyme patterns of heterozygotes are co-dominant, rather than identical with those of either parent; parental patterns are expected in inbred strains (20) (see discussion in miniprint). It therefore seems unlikely that the quantitative differences are determined by the primary structure of the isozymes.

Both qualitative and quantitative variation in pancreatic amylase as represented here by the interstrain differences in patterns of isozyme expression, may be explained by variation in the number of amylase structural genes (20) or by variation at cis-acting sites (see discussion in miniprint). At present we are not able to distinguish between gene-copy and regulation-sequence models. However, the functional variation we have found associated with the amylase gene region in these mouse strains make them ideal material for further molecular analysis.

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MATERIALS AND METHODS

Animals: Breeding pairs of F18C and C3HeB/Fe mice were procured from Dr. Byrne of the University of California, Berkeley. The C3HeB/Fe mice were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. The F18C mice were maintained in our laboratory. In addition to the inbred strains, we have used 1 congenic line with different amylase phenotypes. These lines were maintained at the Institute of Genetics, Albany, New York. The congenic line was maintained by repeated backcrossing to the F18C background. This was accomplished by backcrossing of heterozygotes to F18C females for 4 generations. The hemoglobin condition was then reselected from individuals among the heterozygotes. The procedure used for the genetic cross is the same as that used in our laboratory. The designation C3HeB/Fe, for example, stands for a line in which the amylase complex from the F18C has been transferred to C3HeB/Fe animals. Mice were maintained on a 12-hour light, 12-hour dark schedule and had access to Charles River chow and water ad libitum. Animals between the ages of 2-4 months were used for the experiments.

Enzyme Assay: Enzyme activity was assayed by a modification (1) of the 2,3-diphenyl-5-methyltetrazolium chloride (2) method. Enzyme was assayed in buffer A with a Polytron homogenizer (Irvine Scientific). Activity was assayed from homogenates of high-molecular-weight polyacrylamide beads (15% polyvinylpyrrolidone) as described by Schmauss and Sens (3). The washed glycohydrolases were assayed in 0.4 ml of buffer A and incubated at 37° for 30 min to digest the glycogen. Residual insoluble material was removed by centrifugation. Amylase activity of the purified preparations is plotted in the presence of 0.1% dextrin sevofructose. The assay was terminated with an equal volume of 0.1 M sodium citrate, pH 5.0. The specific activity of these preparations is approximately 1000 units/mg protein.

Preparation of Amylase: Amylase preparations purified from strain (C3HeB/Fe) by the procedure of Rose and Miller (1) was used for the experiments.

Analytical Data: Amylase activity was measured by electrophoretic separation of a material obtained from the amylase complex from the C3HeB/Fe mice. The purified material was then assayed for amylase activity by the procedure of Rose and Miller (1). The specific activity of the purified preparation is approximately 1000 units/mg protein.
Table 1. Quantitative Analysis of Amylase Activity in the Mouse

| Organ Extract | Percent of Amylase Activity Associated with Each Isotype | Percent of Amylase Activity Associated with Each Isotype |
|---------------|-------------------------------------------------------|-------------------------------------------------------|
| CH1.1          | 58 ± 1                                                | 61 ± 1                                                |
| CH2.1          | 44 ± 1                                                | 39 ± 2                                                |
| Heterozygote   | 25 ± 0.5                                              | 26 ± 0.5                                              |
| CH1.1           | 57 ± 0.8                                              | 54 ± 0.8                                              |
| CH2.1          | 36 ± 0.8                                              | 35 ± 0.8                                              |
| Heterozygote   | 60 ± 0.5                                              | 57 ± 0.5                                              |

Table 2. Developmental Synthesis of Amylase Activity in the Mouse

| Organ Extract | Percent of Amylase Activity Associated with Each Isotype | Percent of Amylase Activity Associated with Each Isotype |
|---------------|-------------------------------------------------------|-------------------------------------------------------|
| CH1.1          | 58 ± 1                                                | 61 ± 1                                                |
| CH2.1          | 44 ± 1                                                | 39 ± 2                                                |
| Heterozygote   | 25 ± 0.5                                              | 26 ± 0.5                                              |
| CH1.1           | 57 ± 0.8                                              | 54 ± 0.8                                              |
| CH2.1          | 36 ± 0.8                                              | 35 ± 0.8                                              |
| Heterozygote   | 60 ± 0.5                                              | 57 ± 0.5                                              |

Figure 1. Quantitative Analysis of Amylase Activity in the Mouse. A, B, and C, from purified amylose were separated by electrophoresis as in Figure 1. Cells were stained with bromophenol blue, as in Figure 1, and scanned with a scanning densitometer.

Figure 2. Electrophoresis of purified amylose. The pattern of amylose was visualized by staining with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250.

Figure 3. Developmental analysis of amylose activity in the mouse. A, B, and C, from purified amylose were separated by electrophoresis as in Figure 1. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250.

Figure 4. Immunoprecipitation of the four amylose isoforms. CH1.1 and CH2.1 are from purified amylose. A, B, and C, from purified amylose were separated by electrophoresis as in Figure 1. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250.

Figure 5. Developmental analysis of amylose activity in the mouse. A, B, and C, from purified amylose were separated by electrophoresis as in Figure 1. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250. The tralk was stained with Coomassie Brilliant Blue R-250.