Expression of the Esterase-2 Alleles in *Mus musculus*

by

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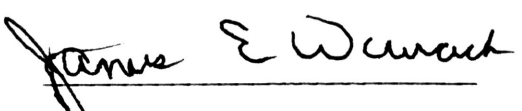

Dr. James E. Womack

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ABSTRACT

Several alleles exist for a polymorphic esterase locus located on chromosome-8 in Mus musculus. The locus, which codes for esterase-2, is expressed as four alleles including an $Es\text{-}2^{A}$ (null) allele exhibiting no protein production and an $Es\text{-}2^{B}$ (normal) allele exhibiting a normal amount of protein production. In order to understand the difference in expression of these alleles it becomes necessary to isolate and translate total cellular mRNA for each. Total cellular RNA was prepared using a guanidine-HCl procedure and applied to an oligo(dt)cellulose column which enabled separation of a poly(A+)-enriched mRNA fraction. Cell-free translation was attempted using mRNA fraction and incorporation of ^{35}S-methionine noted by scintillation counting. Early trials showed no incorporation and no conclusion could be reached on the type of expression involved.

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INTRODUCTION

Chromosome-8 of the house mouse, Mus musculus, has been found to contain loci for at least seven closely related polymorphic enzymes known as esterases. The linkage relationships as well as chromosomal position of these loci have been previously established by standard gene-mapping techniques. Various alleles, differing specifically in protein expression, have been identified through electrophoretic mobilities and their pharmacological properties studied.

Little is known about the biological function of esterases or the cellular control of their expression. Although it has been well established that differences occur in protein expression for the alleles of a specific locus of esterase, little is known about the point at which these mutations are active (i.e., translation or transcription).

The Es-2 locus is of particular importance in gaining information on the nature of these mutations is the Es-2 locus. Four alleles exist: $Es\text{-}2^{B}$ and $Es\text{-}2^{C}$ showing normal protein expression, $Es\text{-}2^{D}$ showing reduced expression, and $Es\text{-}2^{A}$ showing no expression. Because of the presence of a null allele and its inducibility, esterase-2 can easily be used to determine the relationship between transcription, translation, and expression for the esterases. Previous experimentation with esterase-2 has shown it to be specifically induced by testosterone (1), but again no information was gained on the nature of this induction.

Although work in this area indicates that similar mutations commonly function at the transcriptional level, one cannot assume that a translational factor does not exist without studying cellular mRNA levels for the different alleles. By employing biochemical methods to isolate and translate the total cellular mRNA from Es-2^A and Es-2^B strains, the nature of the mutation for these alleles can be determined.

OBJECTIVES

The objectives of these experiments were:

1) to determine the point at which the Es-2 alleles differ during expression;
2) to study the variations in protein production for the Es-2 alleles;
3) to determine the relationship between expression and mRNA production for both the Es-2^A (null) and Es-2^B (normal) alleles.

MATERIALS AND METHODS

Animals

All mice were inbred strains with well established electrophoretic profiles for Es-2. These mice were from the coloney of J. E. Womack, Ph.D., who purchases from Jackson Laboratory, Bar Harbor, Maine. Only male mice were used due to their amplified expression of Es-2 (1).

Preparation of Samples

Approximately two grams of liver were excised from mice and immediately frozen to prevent ribonuclease activity. The sample was then homogenized in 2.7 volumes of 6M guanidine-HCl using a Waring blender, then vortexed for three minutes in a plastic conical tube. After addition of 0.6 ml of 2M potassium acetate, pH 5.0, the sample was vortexed for 10 minutes at high speed, followed by the addition of 2 volumes of 4% Sarkosyl in 0.1M Tris, pH 8.0, and one minute of vortexing. Cesium chloride (7.2g) was added directly to the sample and dissolved by gentle swirling for approximately 30 minutes. 1.6 ml of a cesium chloride pad (5.6 M CsCl in 0.1M EDTA, pH 7.0) was added to a 9/16" x 3.5" polyallomer centrifuge tube. The sample was carefully layered over the cesium chloride pad (total tube weight 17.5g) and centrifuged in an SW41 rotor at 32000 rpm and 20-26°C overnight. The RNA formed a cloudy gelatin-like pellet which was carefully removed with a disposable siliconized pipette. The sample was then dissolved with 2 volumes of cold 95% ethanol. The RNA was

collected by centrifugation and reprecipitated as above. After collection by centrifugation, RNA was dissolved in sterile 1.0mM EDTA. Concentration was determined by U.V. absorption.

Oligo(dT)Cellulose Chromatography of the Total Cellular RNA

Using oligo(dt)cellulose powder obtained from BRL, oligo(dt)-cellulose, chromatography was performed according to Aviv and Leder (2). The typical yield ranged from 20 to 40 ug of poly(A+)-enriched mRNA. These yields were much lower than expected for a 2g sample of liver.

Preparation of mRNA for Cell-Free Translation

The mRNA from above was precipitated with 0.1 volume of potassium acetate, pH 6.5, and 2 volumes of 95% ethanol. After standing overnight at -20% the mRNA was collected by centrifugation in a microcentrifuge and dissolved in sterile, distilled H_2O. The mRNA concentration was determined and the sample lyophilized to remove any ethanol still present. The sample was then redissolved in sterile H_2O and frozen at -70°C until use.

Cell-Free Translation of Poly(A+)-enriched mRNA

Cell-free translation of the mRNA was performed according to Delham and Jackson (3). Using 25 ul reaction mixtures, five trials were performed (Table 1). All endogenous RNA was destroyed by a five minute incubation of the lysate with micrococcal nuclease followed by addition of EGTA. EGTA to chelates the Ca^{++} causing inactivation of

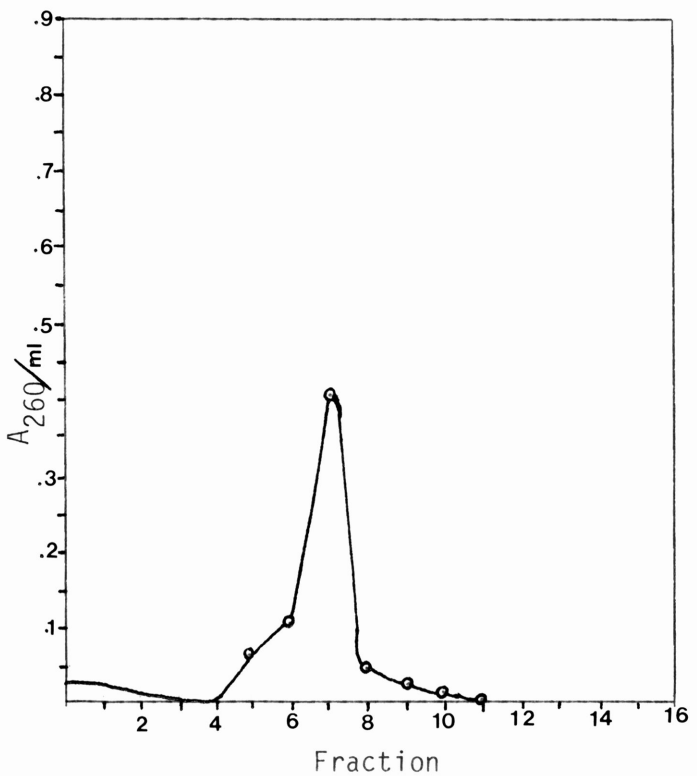
the nuclease. The trails were assayed using scintillation counting. The first trials were performed to insure viable mRNA. Immunoprecipitation assays with specific Es-2 antiserum and starch gel electrophoresis followed by histochemical staining for Es-2 would have been performed on the reaction mixtures of later trials to reach final objectives.

TABLE 1

Reaction Scheme and Counting Results for Cell-Free Translation in the Presence of ^{35}S-methionine

Trial Number	Cocktail	Lysate	^{35}S-Met	mRNA:Count
1	5 ul	10 ul	5 ul	5(H_2O)ul :5065.0
2	5 ul	10 ul	5 ul	5(Es-2^B)ul:2422.0
3	5 ul	10 ul	5 ul	5(Es-2^A)ul:1589.0
4	5 ul	10 ul	5 ul	5(Es-2^B)ul:1691.0
5	5 ul	10 ul	5 ul	5(Es-2^A)ul:1733.0

FIGURE 1



Oligo (dt) Cellulose Chromatography of Total Cellular RNA
(arrow indicates introduction of elution buffer)

RESULTS AND DISCUSSION

Total RNA Preparation

Total RNA was successfully isolated using the guanidine-HCl procedure described earlier. A definite increase in yield was noted over a previously employed method described by Tukey et al. (4). The yield ranged from 11.7 mg to 24.7 mg of total cellular per 2 gram sample RNA with an average yield of 19.0 mg.

Isolation of the mRNA Fraction

In order to study the expression of esterase-2, the need arose for a convenient method of isolating mRNA suitable for later cell-free translation experiments. Oligo(dt)cellulose provided just such a method because of its specificity for the poly(A+) tail found commonly attached to mRNA. After application of the total RNA preparation to the column, thorough washing with binding buffer was carried out. RNA and protein contamination was monitored by U.V. absorption until negligible levels were reached. Upon application of the elution buffer, the eluate was fractionated into 1 ml fractions and the U.V. absorption for each recorded (Fig. 1). Retention data indicates successful isolation of poly(A+)-enriched virtually free of contamination proteins and RNA. Retentaion levels were much lower than expected with an average percentage retention of less than 1%. Typical percentage yields vary from 3 to 10% depending on washing time. Final yeilds of mRNA were very low ranging from 20 to 40 ug (typical yield is ~ 0.1%).

Cell-Free Translation Analysis

Due to time limitations, no significant data was obtained from translation of the mRNA preparation. The results of scintillation counting indicated that no incorporation of ^{35}S-met occurred (Table 1). Obviously, the mRNA has become inactive at some step in its isolation. Upon optimization of the described techniques, viable mRNA could be obtained and decisive data received from study of the reaction mixtures for presence and absence of Es-2. By performing starch gel electrophoresis on the reaction mixtures of Es-2^A and Es-2^B followed by a highly specific histochemical staining for Es-2, a noticeable absence in the null strain versus presence in the normal strain, might be obtained. This data would yield conclusive evidence in determining the nature of Es-2 expression.

CONCLUSION

Although results for both the total RNA prepartion and mRNA isolation were positive in nature, no significant data was obtained from the cell-free translation. Without evidence from studies done on the cell-free translation mixture, no conclusive statement can be made about the nature of the expression for the Es-2 alleles. With increased effectiveness as well as repetition of the techniques, incorporation studies would lead to a well-founded conclusion.

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