either in the cell or during precipitation with 5*M* NaCl in the isolation procedure.

A number of possibilities exist to explain the failure of the final assembly of protein and DNA leading to complete satellite nucleocapsids.

1) It is possible that the satellite proteins produced by herpes complementation are faulty and differ either in amino acid composition or molecular weight from the truly functional capsid proteins produced by adenovirus complementation. This possibility is open to experimentation using polyacrylamide gels.

2) The proteins made in the herpesvirus complementation system are the same as those made in the competent adenovirus complementation system. However, maturation is not just a self-assembly process but rather an adenovirus-controlled satellite virus specific morphopoetic factor. A maturation activating factor, such as that found in the R17 phage system (*13*), or a genetically controlled step, such as the attachment of the fibers to the base plate in the phage T4 (*14*) may be missing in the herpesvirus but present in the adenovirus system. It is also possible that instead of lacking a particular function, herpesvirus may be providing a specific repressor action.

Herpesviruses shut down cell DNA synthesis (*15*), and may therefore turn off some cellular function necessary for satellite maturation. This hypothesis is open to test in a three-component system—herpesvirus inoculation followed by challenge with satellite virus together with helper adenovirus. An active repression step by herpesvirus rather than a paucity of genetic information would be expressed by a failure to replicate satellite virus in this three-component system.

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## Linkage of Genes Controlling the Rate of Synthesis and Structure of Aminolevulinate Dehydratase

Abstract. *The rate of synthesis of δ-aminolevulinate dehydratase in mice is controlled by alleles at the levulinate* (Lv) *locus. Two structural mutations modifying the sensitivity of δ-aminolevulinate dehydratase to heat have been identified. Genetic analyses established that the structural locus for δ-aminolevulinate dehydratase was either physically close to or identical with the locus that controls its rate of synthesis.*

The enzyme δ-aminolevulinate dehydratase (ALD) is involved in the biosynthesis of heme, specifically converting δ-aminolevulinic acid to porphobilinogen. In mice, the activity of ALD is under the control of alleles at the levulinate (*Lv*) locus in Linkage Group VIII (*1–3*). Inbred mouse strains homozygous for the $Lv^a$ allele have approximately three times as much ALD activity per gram of tissue (liver, kidney, and spleen) as do those mice homozygous for the $Lv^b$ allele. These differences in activity are attributable to differing amounts of enzyme protein rather than to any structural changes that modify enzyme activity (*4*). Enzyme purified from the livers of representative $Lv^a$ (AKR/J, DBA/2J) and $Lv^b$ strains (C57BL/6J) is indistinguishable on the basis of all properties studied (*3, 4*). These include electrophoretic mobility, sedimentation coefficient, stability to heat and trypsin, specific activity, $K_m$, and immunochemical criteria. Studies on ALD turnover (*4*) have demonstrated that the *Lv* locus effects its control over enzyme amount by increasing the rate of ALD synthesis two- to threefold in strains of mice homozygous for the $Lv^a$ allele. No effect on the rate of enzyme degradation is apparent.

Genetic control of the rate of enzyme synthesis by a single gene is rare in mammals and the nature of this control can only be explained fully when other genetic loci affecting ALD activity (particularly the structural locus) are identified. This report describes the properties of structurally modified ALD from livers of two inbred strains of mice and establishes that the genetic factor responsible for the structural modification is linked and possibly allelic to the *Lv* locus.

Hutton and Coleman (*2*) classified nearly 30 inbred strains of mice with respect to ALD activity levels. For the most part, all strains fell into three distinct classes: high ($Lv^a/Lv^a$), low ($Lv^b/Lv^b$), and an intermediate class designated as $Lv^c/Lv^c$. This latter class could represent either a third allele at the *Lv* locus or a structural modification of ALD that either increases or decreases the specific activity of the enzyme produced. Thus all strains of mice with atypical enzyme activity levels were considered as potential sources of structural mutations and were each studied carefully with respect to many physical chemical properties, including the rate of heat denaturation.

Enzyme was prepared from the livers of the strains of interest, purified to the ammonium sulfate stage, and compared with that isolated from C57BL/6J mice which served as an arbitrary standard. All enzyme purifications and assays were carried out as previously described (*3*). At 78°C, ALD from the C57BL/6J strain had a half-life of 13.6 ± 0.5 minutes, while enzyme from the C57BR/cdJ strain was somewhat more heat labile ($t_{1/2}$ = 7.7 ± 0.5 minutes). Enzyme from the SM/J strain was considerably more heat resistant ($t_{1/2}$ = 26.7 ± 0.8 minutes) than enzyme from either the C57BL/6J or C57BR/cdJ strains.

The SM/J and C57BR/cdJ strains were chosen for genetic analysis because the differences in the rate of heat

denaturation were the most extreme and because these strains differed at the *Lv* locus, thereby allowing the classification of all offspring with respect to this locus. Typical values for the ALD activity per gram of liver are 5.5 ± 0.2 units for the C57BR/cdJ strain and 1.2 ± 0.1 units for the SM/J strain (*1*, *2*). As expected, the livers from the (C57BR/cdJ × SM/J)$F_1$ hybrids contained intermediate amounts of ALD activity (3.33 ± 0.11 units per gram of liver). The ALD isolated from the livers of these $F_1$ mice on heat denaturation had a halflife of 16.5 minutes, a value intermediate between those of the parental strains (Fig. 1).

In order to obtain the greatest amount of enzyme for future study, the $F_1$ ($Lv^a/Lv^b$) mice were backcrossed to the C57BR/cdJ ($Lv^a/Lv^a$) parental strain. At 60 days of age, the offspring were killed and a portion (0.2 g) of each liver was assayed for ALD activity. As expected, offspring of two classes were produced with respect to ALD activity. Of 82 backcross mice, 42 had intermediate enzyme activity like the $F_1$ parent (3.12 ± 0.13 units per gram of liver) and were classified $Lv^a/Lv^b$, while the remaining 40 mice had high enzyme activity (5.51 ± 0.10 unit/g) indistinguishable from the high-activity parent (C57BR/cdJ) and were classified $Lv^a/Lv^a$. The remaining liver was pooled by class into four groups of ten for enzyme isolation and subsequent heat denaturation studies. A typical heat inactivation analysis is seen in Fig. 1. A straight line was fit to the data by the method of least squares, and the half-life was calculated from the regression equation. Differences between groups were analyzed by means of an analysis of variance and the mean half-life per group was compared by the Student-Newman-Keuls multiple range test.

If the structural locus for ALD was not linked to the *Lv* locus a random selection of mice would have occurred producing pooled enzyme with a heat denaturation rate somewhere in between that seen for the $F_1$ parent and that seen for the C57BR/cd parent. On the other hand, if the structural locus was linked to the *Lv* locus, selection of the mice on the basis of the *Lv* locus would select them also on the basis of the structural locus. This would result in two classes of mice distinguishable both on the basis of enzyme amount and rate of heat denaturation. Figure 1 shows that this

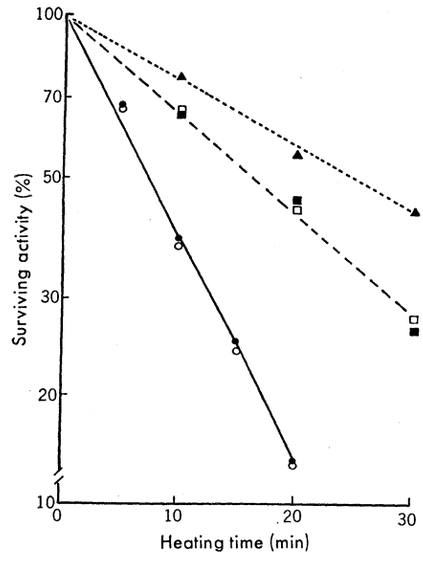


Fig. 1. Heat inactivation (at 78°C) of ALD from livers of SM/J ($Lv^b/Lv^b$) mice, ▲---▲; C57BR/cd ($Lv^a/Lv^a$) mice, ●———●; $F_1$ ($Lv^a/Lv^b$) mice, ■——■; and both classes of backcross mice $Lv^a/Lv^b$, □——□, and $Lv^a/Lv^a$ ○———○. Residual enzyme activity was measured as previously described (*3*).

was the case. All groups classed as $Lv^a/Lv^a$ had an average rate of heat denaturation ($t_{1/2}$ = 7.96 minutes) indistinguishable ($P < .01$) from that observed for the parental $Lv^a/Lv^a$ (C57BR/cdJ, $t_{1/2}$ = 7.81 minutes) strain, while the enzyme isolated from those groups of mice heterozygous at the *Lv* locus had an average rate of heat denaturation ($t_{1/2}$ = 16.9 minutes) indistinguishable from the $F_1$ parent ($t_{1/2}$ = 16.5 minutes). Since the two properties of the enzyme (structure and amount) segregate together, linkage of the genetic factors controlling structure and amount is established and the possibility of allelism is strongly suggested.

If the *Lv* locus is, in fact, the structural locus for ALD, it may be controlling the rate of synthesis of the enzyme only indirectly. For instance, the rate of synthesis may be dependent in one genotype on the availability of a particular amino acid not utilized to as great an extent in the enzyme of the other genotype. However, the best evidence to date regarding their physical and chemical parameters indicates that the enzymes isolated from either of two $Lv^a/Lv^a$ strains (AKR/J, DBA/2J) and the $Lv^b/Lv^b$ strain (C57BL/6J) are identical. Recent data (*5*) extend these findings by demonstrating that ALD from each genotype is composed of six identical subunits, each with one active site. Further, the number of tryptic peptides obtained and the total amino acid composition of each subunit from each genotype is identical. Although the sequence of amino acids in the entire molecule has not been established, it seems unlikely that the enzyme found in these typical $Lv^a$ and $Lv^b$ strains will differ in anything other than their rate of synthesis.

The decreased rate of synthesis in homozygous $Lv^b$ mice could result from a gene dosage effect such that mice containing the $Lv^b$ allele have fewer copies of the dehydratase structural gene. For this explanation to pertain, it would be required that the rate of enzyme synthesis be limited by the number of copies of the structural gene. This hypothesis would provide the simplest explanation for the 3 : 2 : 1 activity levels found in the $Lv^a/Lv^a$, $Lv^c/Lv^c$, and $Lv^b/Lv^b$ genotypes respectively, since these could have six, four, and two copies of the structural gene, respectively. Alternatively, the three genotypes of mice may carry only single copies of the structural gene but have different controlling elements located close to them that regulate some aspect of transcription or translation of the structural gene possibly analogous to that observed in human thalassemia, in which the production of hemoglobin $\beta$ chains is drastically reduced by an adjacent genetic regulating element (*6*). As yet it is not possible to distinguish between these different mechanisms, but it is hoped that further detailed studies on this and other regulating mutants in mammalian systems will clarify our interpretation of the data, thereby leading to a better understanding of some of the different mechanisms of genetic control available to mammalian systems.

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