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The Analysis of Partial Dominance of Intragenic Mutations at the *Adh* Locus in *Drosophila melanogaster* at Two Different Alcohol Concentrations

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Abstract: The dominance of a de novo mutation determines its effect on the viability of the first and succeeding generations; therefore, it is a major factor in estimating the risk from mutations to human health. It is well established that large deletions lead to significant dominance, but until the recent development of molecular methods for amplification and sequencing of mutations it was not possible to determine the dominance of specific intragenic changes in DNA. The purpose of this study was to determine the dominance for a series of *Adh*-null mutations, each with a minimum mutation of a transition at one base pair that produced a single amino acid substitution. This study reports partial dominance of 14 2-CIEMS induced intragenic alcohol dehydrogenase (ADH) null mutations in *Drosophila melanogaster* previously sequenced. Two different alcohol concentrations, 8% and 10%, were used to determine alcohol tolerance. The percentage survival for 72 h was analyzed with 5 replicates for each mutation treatment level. Results showed a broad spectrum of dominance, from high dominance to apparently no dominance. Different sites of mutation had different effects on dominance. Two mutations, nBR140 and nBR149, were not statistically different from wild-type in all 3 statistical tests: (i) untransformed percentage survival, (ii) arcsine transformation and (iii) Johnson & Kotz transformation. Three mutations, nBR139, nBR150 and nBR164, were significantly different from the control at both alcohol concentrations in all statistical analyses.

Key Words: Alcohol dehydrogenase (ADH), *Adh*-null mutations, *Drosophila melanogaster*, partial dominance.

Drosophila melanogaster'in *Adh* Lokusunda Gen İçi Mutasyonların Kısmi Baskınlığının İki Farklı Etanol Konsantrasyonunda Belirlenmesi

Özet: De novo mutasyonların baskınlığı ilk ve sonraki generasyonlarda hayatta kalma oranını belirlemektedir. Bu nedenden ötürü, de novo mutasyonların baskınlığı insan sağlığını etkileyebilecek risklerin tahmininde temel bir faktördür. Büyük delesyonların etkin bir baskınlığa yol açtığı bilinmektedir. Fakat son yıllarda yıllarda mutasyonların amplifikasyonu ve baz dizi tayini yöntemleri ile ilgili moleküler metodların gelişimine kadar DNA da spesifik gen içi mutasyonların dominantlığını saptamak olası değildi. Bu çalışmanın amacı herbiri birtak baz değişikliği sonucunda bir aminiasitin değişmesi ile sonuçlanan bir seri *Adh*-null mutasyonu için değişik baskınlık derecelerinin belirlenmesidir. Bu çalışmada daha önce Fosset ve ark. (1) tarafından baz dizi tayini yapılan 2-CIEMS kullanılarak oluşturulmuş olan gen içi alkol dehidrogenaz (ADH) null mutasyonlarındaki kısmi baskınlık rapor edilmektedir. Alkol toleransını belirlemek amacı ile %8 ve %10'luk olmak üzere iki farklı alkol konsantrasyonu kullanılmıştır. Herbir mutasyon için 5 tekrar yapılmış olup bu tekraralarda 72 saat içindeki % yaşama oranları kaydedilmiştir. Sonuçlar yüksek baskınlık derecesinden, hiç baskınlığın olmadığı duruma kadar geniş bir spektrumda dağılım göstermiştir. Mutasyonların ge içindeki buldukları bölgeler, baskınlığa değişik şekilde etki etmiştir. Bütün istatistiksel analizler, (i) transfor edilmemiş % yaşama oranı (ii) arc-sine transformasyonu ile (iii) Johnson-Kotz transformasyonu ile, sonucunda nBR140 ve nBR149 stoklarının kontrolden istatistiksel olarak farklı bulunmamıştır. Diğer taraftan nBR139, nBR150 ve nBR164 stokları her iki alkol konsantrasyonu için 3 istatistiksel analiz sonucunda da kontrol grubundan farklı bulunmuştur.

Anahtar Sözcükler: Alkol dehidrogenaz (ADH), *Adh*-null mutasyonları, *Drosophila melanogaster*, kısmi dominantlık.

Introduction

Transmissible de novo mutations in the germ line usually produce null alleles that are recessive. Typically, the null alleles at any essential locus will have a low

frequency of less than 1% due to the selection against its detrimental phenotype. With little inbreeding, the probability of a de novo autosomal mutation is about 1/q times more likely to be in a heterozygote than in a

homozygote. For example, with q less than 1%, the probability of the mutant being homozygous is less than 10^{-4} . Therefore, the risk assigned to recessive autosomal mutations largely depends on the expression of the mutation in the heterozygote. The effect of a de novo mutation for risk assessment depends on the departure of the heterozygote phenotype from the homozygous dominant normal phenotype. Dominance will be the main determinative for risk assessment and has the greatest effect on the first generation with decreasing effects in successive generations.

Heterozygous effects of chemically induced (ethyl methanesulfate (EMS)) mutations have been studied (1-6). It was reported by Temin (6) that there was a small but statistically significant reduction in the viability of flies that are heterozygous for the EMS treated second chromosome. In addition, the heterozygous effects of X-ray induced mutations have been studied (7-13). The radiation induced lethals had a 4-5% decrease in the viability of heterozygotes (14). The combined results from these experiments demonstrate the importance of the effects of dominance on viability and, more significantly, on fitness.

The *Drosophila* alcohol dehydrogenase locus (*Adh*) is a very useful model for mutation studies because it possesses several advantageous properties: (a) naturally occurring mutations and those induced by mutagens can be sequenced because the introns are small, (b) the enzyme is so abundant in a single fly that the Alcohol dehydrogenase (ADH) activity can be easily measured and (c) the electrophoretic variants of ADH are available, and for ADH hybrids between *AdhF* and *AdhS* (for fast and slow migration on SDS page) the peptide homodimers for F and S can be distinguished from each other and also from the heterodimer F/S. Therefore, flies with an interesting *Adh* mutation (s) can be crossed with mutant or wild-type flies to understand the properties of the hybrid molecules (15-18).

The function of the *Drosophila* ADH enzyme (alcohol: NAD oxidoreductase EC 1.1.1.1) is the detoxification and utilization of environmental alcohols. Regulation of ADH expression in *Drosophila* was studied in detail (15). This enzyme can reversibly catalyze the conversion of alcohols to their oxidation products such as aldehydes (from primary alcohols) and ketones (from secondary alcohols) (15). Ketones are usually toxic to animals and metabolically inert. Almost 90% of the total ethyl alcohol,

which is the most important alcohol in the environment, in wild-type *Drosophila* is degraded by ADH (17). There is a positive relationship between ADH activity and ethanol tolerance (19, 20). Flies carrying a null mutant allele at their *Adh* locus are more sensitive to the toxic effects of ethanol than the normal wild-type flies reviewed by Geer, Heinstra and McKechnie (17).

The active ADH is a dimer of consisting 2 subunits, each with a molecular weight of 27,400, which are transcribed from a single copy of the gene *Adh*. If both alleles are being transcribed in null/normal heterozygotes, a heterodimer and a homodimer for the mutant allele and a homodimer for the normal allele should be produced, assuming that the mutant allele produces a peptide capable of dimer formation. The catalyzing efficiencies of some of these heterodimers may be lower than that of the control F/S heterodimer and some may be near the lower limit of detectability (16).

It is well established that large deletions lead to significant dominance (21) but intragenic changes in DNA could not be studied until the recent development of molecular methods for the amplification and sequencing of DNA. It is now possible to determine the dominance of specific intragenic changes in DNA. In this experiment, partial dominance at the *Adh* locus, when heterozygous with wild-type, of 14 intragenic recessive null mutations was studied. These mutations were induced with 2-chloroethylmethanesulphate (2-CIEMS). These mutants were sequenced and all were due to single base pair GC to AT transition. These intragenic *Adh*-null mutants were introduced into a wild-type *AdhF* allele (location 50.1) flanked by black (*b*) (location 48.5) and purple (*pr*) (54.5) on the second chromosome. This chromosome was made isogenic and then homozygous *b AdhF pr* by crossing to stocks that contained inversions to suppress crossing over and both dominant and recessive marker genes. This *b Adh pr* homozygous chromosome was expanded and constituted the control of the stock from which all male second instar larvae were treated with 2-CIEMS; therefore, all cis acting modifiers and promoters were the same for both treated and control chromosomes. The partial dominance of *Adh*-null mutations with single base pair substitutions was measured by alcohol tolerance at 2 different alcohol concentrations: 8% and 10%. These values are equal to the environmental stress that can be found on seepage from wineries (22). This high selective stress may enhance the role of *Drosophila* ADH in alcohol tolerance.

Materials and Methods

(i) *Drosophila* stocks

Fourteen 2-CIEMS induced *Adh*-null mutants (Table 1) (named Adh^{nBR} and induced from *Adh^F* stocks), heterozygous with *CyO* (*In (2LR) O, Cy dp^{lv1} Adh^{nB} pr cn*), used in this experiment were sequenced by Fossett et al. (1). The *CyO, In (2LR) O*, has multiple inversions that prevent crossing over, and is marked with a dominant gene, *Cy*, for curly wings and a recessive lethal dp^{lv1} that prevents this chromosome from being homozygous. All stocks and control (wild-type) were kept at 25 °C on standard corn meal containing agar, corn sugar and brewers yeast media with propionic acid to inhibit mold.

(ii) Genetic crosses

The control and mutagenized second chromosome, *b Adh^F pr*, was outcrossed 3 times to make a nonmutagenized genetic background, except for chromosome 2, which was prevented from crossing over by inversions on the balancing chromosome. Young virgin females, *b Adh pr/CyO, In (2LR) O, Cy dp^{lv1} Adh^{nBR} pr cn*, were collected from the null mutant *Adh* stocks and aged to 5-6 days on standard media to confirm virginity by the inability to produce progeny. Later, these virgins were crossed with 5-6 day-old wild-type healthy males from an Oregon R strain that has been maintained in W. Lee's laboratory for approximately 25 years. In each cross almost 50 healthy females were crossed with approximately 25 males.

The F_1 ratio of curly wings to normal flies was not significantly different from 1:1. Curly wing flies were discarded and normal active males, without body damage, were collected. ADH activity was measured only in males because females have a larger variance, probably due to the formation of variable amounts of eggs (20). These normal males were aged at 25 °C on standard media without alcohol and active yeast for 5-6 days, before they were used in alcohol tolerance tests.

(iii) Alcohol tolerance test

The alcohol tolerance test was carried out in 250 ml milk bottles on *Drosophila* media formula 4-24 (from the Carolina Biological Supply Company) at 2 different alcohol concentrations: 8% and 10%. In the 8% alcohol test 5.05 ml of ethanol (95%) was mixed with 35 ml of distilled water and poured over 40 ml of instant *Drosophila* media in the bottle. This gave a volume of 60

ml with alcohol uniformly mixed. In the 10% ethanol test, 6.31 ml of ethanol (95%) was mixed with 35 ml of distilled water and poured on the 40 ml of instant *Drosophila* media for a final volume of 60 ml with 10% alcohol uniformly mixed.

For each stock, at both ethanol concentrations, at least 5 repeats were performed with 250-300 flies tested. Approximately 50 flies were put into each bottle and survival rates were recorded at 24, 48 and 72 h intervals. The alcohol in the media evaporated after approximately 3 days: thereafter, the survival ratios of the flies became stable. For this reason, the percentage survival following 72 h exposure to alcohol or no alcohol for the control was analyzed using SAS version 6.0 software (SAS Institute Inc., Cary, North Carolina, USA). Since the data based on percentages are not normally distributed, it was arcsine square root and Johnson and Kotz (23) transformed prior to analysis. Then, the data were analyzed using one-way ANOVA. When ANOVA indicated significant differences among the survival ratios, Dunnett's procedure was used to determine whether the survival of a genotype was significantly different from the control.

Results

Untransformed percentage survival, arcsine transformed and Johnson & Kotz transformed percentage survival ratios of null/normal heterozygous flies at 8% and 10% alcohol concentrations are presented in Tables 1-3, respectively. With the Johnson and Kotz transformation, 12 of the 14 *Adh*-null mutations, heterozygous with wild-type chromosomes from stock, *Org. R.*, had a phenotype with significantly lower viability than the wild-type control (*Adh^F/Adh^F pr*) at either or both ethanol concentration. At both alcohol concentrations and in all statistical analyses, mutations nBR149 and nBR140 were not significantly different from the wild-type control. Mutations nBR139, nBR150 and nBR164 were found to be significantly lower than the control for both alcohol concentrations and all 3 statistical tests. Mutation nBR142 was significantly different from the control in the 8% alcohol tests for all 3 statistical analyses but not in any 10% alcohol tests.

In the 8% alcohol test, the same 5 mutants were significantly different from the control (at probability of type I error = 0.05) in untransformed percentage

Table 1. Data analyses of 14 2-CIEMS induced *Adh*-null mutants at 2 different ethanol levels with untransformed percentage survival analyses. *Significance level for 8% EtOH test is 54.061. ^ Significance level for 10% EtOH test is 56.781. ~Percentage differences from the significance level in these statistical analyses. (-) indicates that the mutant is not significantly different from the control. (+) indicates that the mutant is significantly different from the control.

Mutant number	Mutation sequence	Amino acid replacement	Significant difference from the control	
			At 8% EtOH tolerance test *untransformed % survival analyses	At 10% EtOH tolerance test ^untransformed % survival analyses
nBR137	ATC ACC CGC T	T186I	~5.94	(+)
nBR138	AGC TCC CTG T	S166F	(+)	~6.21
nBR139	AAG GCC GCC T	A158V	(+)	(+)
nBR140	TCC GGC ACC A	G155D	(-)	(-)
nBR142	AAT GCC ATC T	A146V	(+)	(-)
nBR143	CCC GGC ATC A	G184D	(-)	(-)
nBR147	CTG GGA GGC A	G17R	(-)	(+)
nBR149	AAC GGA GCT A	G93E	(-)	(-)
nBR150	TAC TCC GGC T	S154F	(+)	(+)
nBR153	CC ATG TCG A	Mutation at start codon	(-)	(-)
nBR161	GCC GCC GTC T	A159V	(-)	~9.4
nBR162	CTG GGA GGC A	G17E	(-)	(-)
nBR163	GTT GCC GGT T	A14V	(-)	(+)
nBR164	GGA TCC GTC T	S140F	(+)	(+)

survival and arcsine transformation analysis. However, this figure was 7 in Johnson and Kotz transformed data analysis. Mutation nBR138 has a phenotype under the significance level in all 8% survival analyses but not in 10% untransformed percent survival tests. In this analysis it is just above the significance level with a 6.21% probability. Mutation nBR161 was just above the significance level at 9% probability using the 10% untransformed survival analysis. Mutation nBR137 was slightly above the significance level, using the untransformed survival and arcsine transformation analyses, at 5% and 16%, respectively.

In the 10% alcohol tests, 6, 8 and 11 mutants were significantly different from the control, using the untransformed percentage survival, arcsine transformation and Johnson and Kotz transformed

statistical analysis, respectively. When the selective stress was raised to 10% ethanol level, mutants nBR147, nBR163 and nBR137 were significantly lower than the control.

Discussion

Differential survival of 14 different 2-CIEMS induced intragenic *Adh*-null mutants, single base substitutions with GC to AT, was studied for alcohol tolerance on ethanol supplemented food. Genetic crosses were performed to give a common genetic background for the control and mutagenized chromosomes. The probability of a mutation in the *Adh* locus following exposure to 2-CIEMS was one per thousand. The probability of an independently induced modifier of the *Adh* locus with a

Table 2. Data analyses of 14 2-CIEMS induced *Adh*-null mutants at 2 different ethanol levels with arcsine (a.s.) transformed percentage survival analyses. *Significance level for 8% EtOH test is 0.523. ^ Significance level for 10% EtOH test is 0.908. ~Percentage differences from the significance level in these statistical analyses. (-) indicates that the mutant is not significantly different from the control. (+) indicates that the mutant is significantly different from the control.

Mutant number	Mutation sequence	Amino acid replacement	Significant difference from the control	
			At 8% EtOH tolerance test *a.s. transformed % survival analyses	At 10% EtOH tolerance test ^a.s. transformed % survival analyses
nBR137	ATC ACC CGC T	T186I	~16.8	(+)
nBR138	AGC TCC CTG T	S166F	(+)	(+)
nBR139	AAG GCC GCC T	A158V	(+)	(+)
nBR140	TCC GGC ACC A	G155D	(-)	(-)
nBR142	AAT GCC ATC T	A146V	(+)	(-)
nBR143	CCC GGC ATC A	G184D	(-)	(-)
nBR147	CTG GGA GGC A	G17R	(-)	(+)
nBR149	AAC GGA GCT A	G93E	(-)	(-)
nBR150	TAC TCC GGC T	S154F	(+)	(+)
nBR153	CC ATG TCG A	Mutation at start codon	(-)	(-)
nBR161	GCC GCC GTC T	A159V	(-)	(+)
nBR162	CTG GGA GGC A	G17E	(-)	(-)
nBR163	GTT GCC GGT T	A14V	(-)	(+)
nBR164	GGA TCC GTC T	S140F	(+)	(+)

similar mutation frequency would depend on the number of genes modifying the *Adh* locus, and would be considerably less than one per thousand, thereby permitting a comparison between the induced mutation and the control wild-type allele. The differential survival of *Adh* genotypes on food containing ethanol has been studied previously (20,24-27). Differences in in vitro ADH activities between *Adh* genotypes have also been reported (16,28-30).

There is a positive correlation between ADH activity and survival on food supplemented with ethanol since flies that have higher ADH activity can detoxify the harmful effect of ethanol faster. Moreover, other biochemical relations may be involved in ADH activity. Glycerol-3-phosphate oxidase (GPO, locus *Gpo*) and sn-

glycerol-3-phosphate dehydrogenase (GPDH, locus *Gpdh*) play an important role in alcohol or alcohol product metabolic pathways (17). The function of these minor pathways could be particularly important to the individual when the alcohol concentration is high.

Several experiments have been conducted on null/normal heterodimer formation (16,18,31-35). A mechanism was suggested for the interaction of a recessive null allele and the wild-type monomer in a heterozygote fly; a defect in the folding of a mutant subunit could be corrected by association with a complementing subunit. In this situation, the effect of a mutation should be localized so that a subunit which is correctly folded can restore the active configuration to the mutant subunit (18,31,34,35).

Table 3. Data analyses of 14 2-CIEMS induced *Adh*-null mutants at 2 different ethanol levels with Johnson and Kotz (J&K) transformed percentage survival analyses. *Significance level for 8% EtOH test is 0.752. ^ Significance level for 10% EtOH test is 0.716. ~Percentage differences from the significance level in these statistical analyses. (-) indicates that the mutant is not significantly different from the control. (+) indicates that the mutant is significantly different from the control.

Mutant number	Mutation sequence	Amino acid replacement	Significant difference from the control	
			At 8% EtOH tolerance test *J&K transformed % survival analyses	At 10% EtOH tolerance test ^J&K transformed % survival analyses
nBR137	ATC ACC CGC T	T186I	(+)	(+)
nBR138	AGC TCC CTG T	S166F	(+)	(+)
nBR139	AAG GCC GCC T	A158V	(+)	(+)
nBR140	TCC GGC ACC A	G155D	(-)	(-)
nBR142	AAT GCC ATC T	A146V	(+)	(-)
nBR143	CCC GGC ATC A	G184D	(-)	(+)
nBR147	CTG GGA GGC A	G17R	(-)	(+)
nBR149	AAC GGA GCT A	G93E	(-)	(-)
nBR150	TAC TCC GGC T	S154F	(+)	(+)
nBR153	CC ATG TCG A	Mutation at start codon	(-)	(+)
nBR161	GCC GCC GTC T	A159V	(+)	(+)
nBR162	CTG GGA GGC A	G17E	(-)	(+)
nBR163	GTT GCC GGT T	A14V	(-)	(+)
nBR164	GGA TCC GTC T	S140F	(+)	(+)

It was reported by Hollocher and Place (34,35) and Chenevert et al. (18) that in *Drosophila* EMS induced ADH mutants Gly17 to Arg, Gly93 to Glu, Gly184 to Asp are inactive but form stable homodimers, as well as heterodimers with wild-type ADH, in which the wild-type subunit retains full enzyme activity.

The Gly14 and Gly17 residues are located in the AMP binding domain, a moiety of NAD⁺ (Figure 1) and this region consists of an α - β - α structure. In ADH there is a glycine motif, Gly-Xa₂-Gly-Xa-Gly, in this region. However, in most of its homologues this part of the enzymes contains a Gly-Xa₃-Gly-Xa-Gly motif. The turn is sharper in ADH and glycine residues provide more flexibility for the enzyme and facilitate the close contact with AMP (18,36,37). It was reported that mutating Gly14 to Val almost inactivates the *Drosophila* ADH (36).

In our experiment, stock nBR163, which has Gly14 to Val substitution, was significantly different from the control in the 10% alcohol test in all statistical analyses. This was probably because of the additional carbon group in the valine, which may have disrupted this tight turn. Gly-17 was substituted with 2 different amino acid residues, mutations nBR147 residue Gly17 to Arg and mutations nBR162 residue Gly 17 to Glu. Replacement of glycine with arginine gave significantly different results from the control in all 10% alcohol test survival analyses (Tables 1-3). This may be the result of arginine interfering with the flexibility of this region and adding a positively charged side chain to this part of the ADH. These changes may lead to the loss of interaction with the coenzyme NAD⁺ and the enzyme. When the glycine was replaced with glutamic acid at this site, a less severe effect was

observed on the enzyme activity. Stock nBR162 is significant only at 10% Johnson and Kotz analysis.

Glycine 93 is located in a highly conserved hydrophobic β -strand, which is close to the NAD⁺ binding region (Figure 1). It was reported by Chenevert et al. (18) that the replacement of Gly by a negatively charged Glu at this position probably disrupts the hydrophobic β -strand that binds to NAD⁺. It was also stated by Hollocher and Place (34,35) that interallelic complementation of EMS mutant *Adh*ⁿ¹, a mutant of Gly⁹³ to Glu, can form a heterodimer with *Adh*^s that has half the activity of the wild-type heterodimer. In our experiment, Gly93 to Glu substitution, in nBR149, was not significantly different from the control at 2 different ethanol levels and in all statistical analyses.

Spanning positions 139-158 comprise 2 amino acids, Tyr152 and Lys156. Conserved in all short chain dehydrogenases, these are probably in the substrate

interactive domain and have an important functional role in catalysis (36,39). Interactions between NAD⁺ and Tyr153, Lys157 and Thr186 are important in stabilizing the conformation that allows the substrate to interact with the nicotinamide group (40,41). In this study, replacement of the amino acids near this region, amino acids 154 and 155, gave significant results. Mutation nBR150 residue 154, Ser to Phe, was significantly different from the control in all statistical analyses with 2 alcohol concentrations. Probably, Phe is a larger amino acid than Ser and this may cause the disruption in the configuration of the active site. However, no effect on enzyme activity was observed on mutation nBR140 with residue 155, Gly to Asp.

As can be seen in Figure 2, Ala158 and Ala159 are located in the dimer interface and they form a hydrophobic anchor in this region. The 4 Ala residues from the dimer fit into a hydrophobic tetrahedral

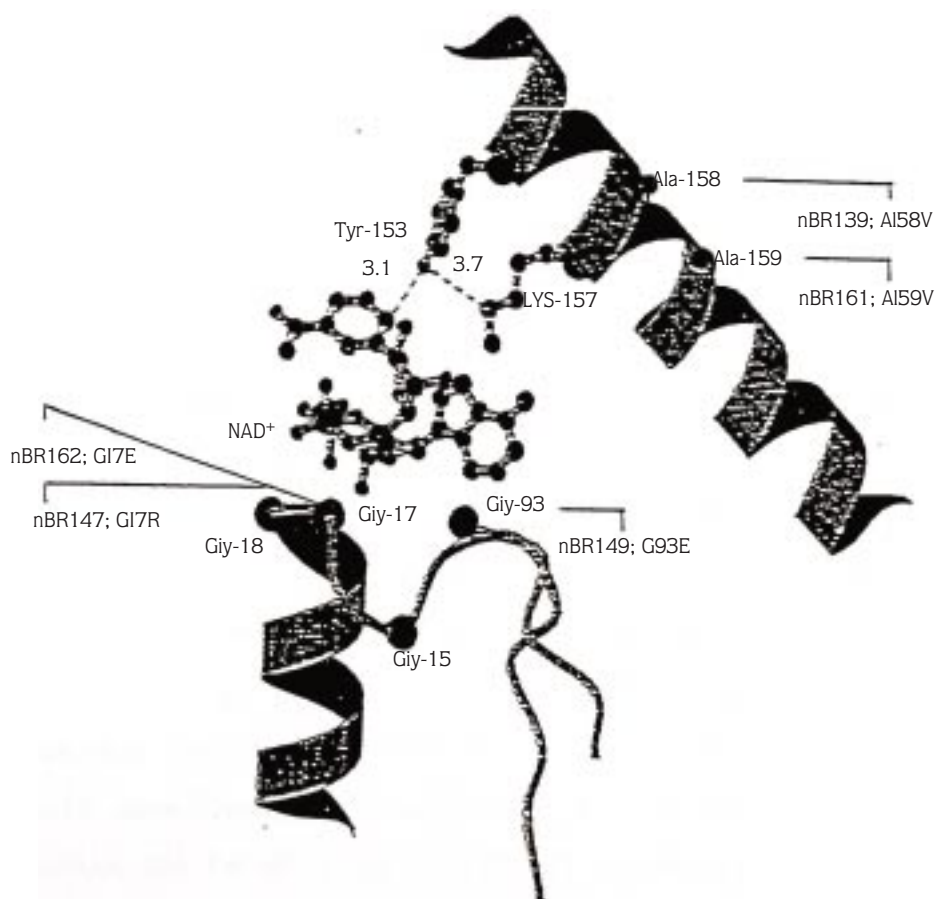


Figure 1. 3D model of dimer interface of *Drosophila* ADH modified from Chenevert et al. (7).

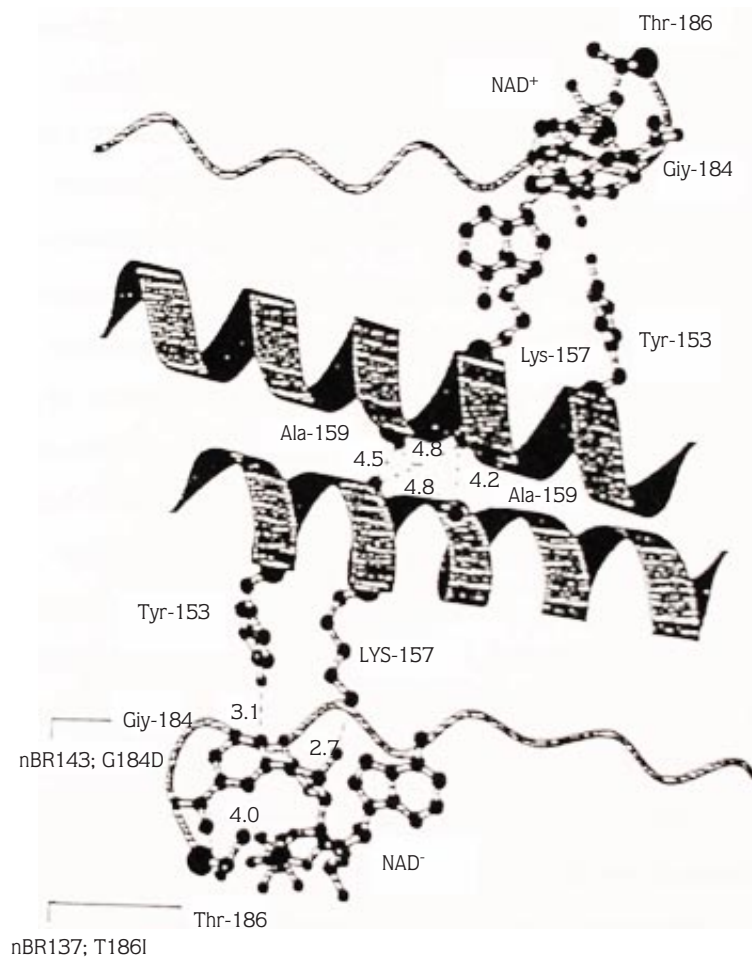


Figure 2. 3D model of the dimer interface in *Drosophila* ADH modified from Chenevert et al. (1995).

configuration, and this is important in stabilization of the ADH dimer. In addition, a substitution in Ala159 with a larger amino acid may alter the conformation and displace either the Lys157 and/or Tyr153 at the catalytic site, which leads to loss of enzyme activity (18). It was reported by Hollocher and Place (35) that the EMS mutant of *Adh*ⁿ², which has Ala159 to Thr substitution, does not form dimers nor interallelic complementation with either *Adh*^S and *Adh*^F that leads to heterodimers. This shows that the Ala159 to Thr mutation affects the stability of functional dimers, leading to loss of enzyme activity. In our experiment, mutations with residue 158, Ala to Val, stock nBR139, and with residue 159, Ala to Val, stock nBR161, showed very important differences from the control in all statistical analyses (Tables 1-3).

The replacement of alanine 158 and alanine 159 with valine residues probably disrupted the configuration and hydrophobicity of this part. Since valine has 1 more carbon atom than alanine, this carbon atom may increase the distance between 2 subunits and reduces the strength of the hydrophobic attraction in this part of the enzyme.

Gly184 is important in the close approach of Thr186 to NAD⁺ ring (Figure 3) and it is conserved among the members of this protein superfamily. It was stated by Jiang et al. (16) that residues from 182 to 194 are not critical to the monomer contact and binding. In addition, Hollocher and Place (35) reported that EMS induced *Adh*ⁿ⁷, which is Gly184 to Asp mutant forms homodimers which are inactive. Moreover, a cross of this mutant with *Adh*^F and *Adh*^S leads to an active heterodimer formation.

Replacement of Gly184 with aspartic acid, in mutation nBR143, was significant only at 10% probability level using the Johnson and Kotz statistical method. The replacement of 186 threonine with isoleucine, in mutant nBR137, almost inhibits the enzyme activity in all statistical tests at 8% and 10% alcohol tolerance levels (Tables 1-3).

In our experiment, we have observed significant differences from the control in the heterozygote with mutations nBR164, residue 140 Ser to Phe, and nBR138, residue 166 Ser to Phe, in all ethanol levels and experimental analyses. More experiments are necessary to clarify the exact functions of these residues.

In this experiment, for each mutation 250-300 flies were used in 5 repeats. With such a large number of flies it was easy to observe the dominance effects of mutations. In heterozygotes, 12 of the 14 mutants showed significant dominance at the 8% or 10% ethanol tests using the Johnson and Kotz transformation.

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