Alcohol Dehydrogenase of Drosophila: Interconversion of Isoenzymes

Abstract. *Isoenzymes of alcohol dehydrogenase extracted from* Drosophila melanogaster *are interconvertible and can be distinguished by electrophoretic mobility. When adsorbed on diethylaminoethyl cellulose, the faster-moving forms are converted to the slowest-moving form; the latter is converted to the former in the presence of 0.05 molar nicotinamide-adenine dinucleotide, and the conversion is accompanied by the binding of 3.5 moles of the dinucleotide per mole of enzyme. A change in heat stability accompanies the conversion of the slowest form of alcohol dehydrogenase to the fastest form; the latter becomes stable at 45°C. The increased heat stability may indicate that a conformational change in the alcohol dehydrogenase occurs along with the binding of nicotinamide-adenine dinucleotide.*

Alcohol dehydrogenase (ADH) of *Drosophila melanogaster* exists in several forms separable by electrophoresis. Genetic analyses have shown that these forms are controlled by a single genetic locus on the second chromosome (*1, 2*). The multiple forms of the enzyme, therefore, may be derived from a single form by binding a ligand, or by conformational changes, or both. I now report that the electrophoretic mobility and the thermal stability may be altered experimentally, and that conversion from the slow to the fast electrophoretic form of the enzyme is accompanied by binding nicotinamide-adenine dinucleotide (NAD) to the enzyme.

A homogeneous preparation of alcohol dehydrogenase was isolated from adults of *D. melanogaster* (Samarkands) by *n*-butanol treatment of a homogenate, fractionation with ammonium sulfate, chromatography on diethylaminoethyl (DEAE)-cellulose and then on hydroxylapatite, and again by fractionation with ammonium sulfate. Polyacrylamide-gel electrophoresis (*3*) and ultracentrifugation show that isolated ADH is homogeneous and consists only of ADH_5. The three electrophoretic forms normally observed in a homogenate are designated ADH_1, ADH_3, and ADH_5 from the fastest to the slowest migration rate. Two other bands, ADH_2 and ADH_4, also exist, but at much lower concentration than the other three forms.

The first evidence of interconversion of the different forms of the enzyme appeared during chromatography. Operation of the column at a relatively slow rate (0.1 ml cm^{-2} min^{-1}) resulted in recovery of the enzyme in high yield (> 90 percent based on quantitative spectrophotometric analysis) in the form of ADH_5 only; ADH_5 represents less than 50 percent of the initial total activity. When the enzyme was eluted more rapidly, activity was distributed among three peaks corresponding to ADH_5, ADH_3, and ADH_1 eluted in this order. Thus, ADH_1 and ADH_3 were converted to ADH_5 when they were adsorbed by DEAE-cellulose for sufficient time.

The reverse interconversion, ADH_5 to ADH_1, was first attempted by including NAD in the polyacrylamide gel during electrophoresis of the enzyme, but no clear conversion was seen (*2*). Ursprung and Carlin (*4*) demonstrated that treatment of *Drosophila* homogenates with NAD for several days or weeks increased the amount of the faster-moving forms; the recovery of the enzyme activity was quite variable. I explored conditions for converting ADH_5 (isolated from the DEAE-cellulose chromatogram) to ADH_3 and ADH_1. This conversion was observed after ADH_5 was exposed to NAD, but was not observed in the absence of this cofactor. The ADH_3 appears first and is followed much later by ADH_1. The rate of conversion of ADH_5 to either faster form is affected by temperature and NAD concentration (Table 1).

Table 1. Conversion of ADH_5 of *Drosophila* to ADH_3 and ADH_1. The ADH_5, purified by chromatography on DEAE-cellulose, was dissolved in 0.05*M* tris (adjusted to *p*H 8.6 at 25°C with H_3PO_4) and dialyzed against ten volumes of the above NAD concentrations in the same buffer. Samples were removed periodically for electrophoresis. When one-third or more of the enzyme appeared as ADH_3 or ADH_1, the time was recorded. Recovery was calculated from spectrophotometric assays performed in 0.1*M* glycine (*p*H 9.5), 0.08*M* 2-butanol, and 2.3 m*M* NAD at 25°C; under these conditions, there is a linear relationship between enzyme concentration and absorbance change at 340 mμ per minute.

Temperature (°C)	NAD (*M*)	Conversion time		Total recovery (%)
		ADH_3	ADH_1	
4	0.001	> 23 days	> 23 days	84
4	.01	4 days	23 days	76
22	.02	2 hours	23 hours	87
22	.05	2 hours	5 hours	85

Another distinguishing property for the different forms of alcohol dehydrogenase is heat stability (*2*). After electrophoresis of either a homogenate or ADH_5 alone, the ADH_5 is inactivated by warming the gel to 45°C for 30 minutes before the enzyme is assayed. The ADH_3 and ADH_1 are not inactivated by this procedure. The ADH_1 produced from ADH_5 by treatment with NAD was found to be heat stable. Thus, the ADH_1 derived from ADH_5 resembles ADH_1 in homogenates in respect to electrophoretic mobility and heat stability.

The ADH_1 resulting from treatment with NAD was then analyzed for bound NAD. After incubating ADH_5 with 0.05*M* NAD at *p*H 8.6 for 6 hours at 23°C, I dialyzed the enzyme against 0.2*M* NaCl in 0.05*M* tris phosphate (*p*H 8.6) to remove unbound NAD. The NaCl was removed by dialysis against buffer alone. The enzyme, now > 90 percent ADH_1, was denatured with 5-percent trichloroacetic acid which was removed by extraction with ether. The amount of NAD in the aqueous phase was determined fluorometrically by forming the methyl ethyl ketone adduct (*5*).

In order to distinguish NAD from fluorescent impurities, I reduced the samples by using yeast alcohol dehydrogenase and ethanol and then calculated the NAD concentration from the fluorescence lost by such treatment. The analyses showed 0.051 μmole of NAD per milligram of protein for one preparation of ADH_1 and 0.066 μmole for another preparation of ADH_1. The molecular weight of *Drosophila* alcohol dehydrogenase appears to be 60,000 (*6*). Thus, there are 3.5 moles of NAD per mole of ADH_1; analyses of ADH_5 revealed less than 0.1 mole of NAD per mole of enzyme.

An independent estimate of the difference in charge between ADH_5 and ADH_1 was obtained by comparing their electrophoretic migrations with those of the three human hemoglobins A, S, and C. In the β^6 position, these hemoglobins contain glutamic acid, valine, and lysine, respectively. Since the subunit structure of hemoglobin is $\alpha_2\beta_2$, A and S differ by two charge units; A and C differ by four charge units. Human hemoglobin and *Drosophila* alcohol dehydrogenase have molecular weights

of 68,000 and 60,000, respectively; hence, each should be similarly restricted by the dimensions of the pores in the gel. The hemoglobins and the *Drosophila* homogenate were both placed on 5-percent polyacrylamide, and electrophoresis was performed (*3*) in 0.05*M* tris (adjusted to *p*H 8.6 at 25°C with H_3PO_4) at 18 volt/cm for 3 hours. Hemoglobin was stained with amido black; alcohol dehydrogenase was located by the reduced tetrazolium procedure (*1*). The distance (in millimeters) from the origin for hemoglobins A, S, and C was 62, 47, and 32, respectively; for *Drosophila* ADH_1, ADH_3, and ADH_5, it was 65, 51, and 31, respectively. Comparative hemoglobin migrations show that a single charge-unit difference would result in a migration difference of 7.5 mm. The migration difference between ADH_1 and ADH_5 corresponds to 4.5 charge units. In that NAD would contribute one charge unit in the oxidized form but would contribute two charge units in the reduced form, the correlation of 3.5 NAD and 4.5 charge units is not unreasonable.

Multiple forms of enzymes that are the result of chemical modification of the basic protein have been observed. Malate dehydrogenase from mitochondria exists in five forms that are interconvertible by treatment with iodine or acid (*7*). Lactate dehydrogenase of the muscle (LDH_5) consists of subbands that can be interconverted by mercaptoethanol (*3*) or formaldehyde (*8*). Sodium borohydride treatment of an esterase (from maize) causes the appearance of enzyme species with electrophoretic migration rates similar to those of naturally occurring isoenzymes (*9*). Alcohol dehydrogenase of horse liver can be altered in electrophoretic migration by treatment with NAD (*10*). Alcohol dehydrogenase of *Drosophila* also may be altered by NAD treatment to a form with both a different electrophoretic mobility and a different heat stability. The increased stability may indicate that a conformation change in protein structure occurs when ADH_5 binds the NAD.

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Titration Patterns of a Murine Sarcoma–Leukemia Virus Complex: Evidence for Existence of Competent Sarcoma Virions

Abstract. *Stocks of murine sarcoma virus show titration patterns ranging from one- to two-hit kinetics. The comparison of various titrations of this virus, both with and without added helper virus, to theoretical model systems composed of defined constituents, suggests the existence of a sarcoma virus that does not need coinfecting murine leukemia virus to be manifested as a focus-forming unit. The behavior of such nondefective particles is compatible with a postulated leukemia-sarcoma virus hybrid.*

Harvey (*1*) and Moloney (*2*) have reported the isolation, from passage lines of the Moloney leukemia virus, of agents that are sarcomagenic for mice, rats, and hamsters (*3*). The Moloney isolate of the murine sarcoma virus (MSV) has been shown to be capable of inducing foci of morphologically altered cells in infected mouse (*4*) and rat (*5*) cell cultures and can thus be readily titrated in vitro. The titration pattern of some stocks of MSV indicated that the infection of mouse cells was defective and required simultaneous infection with a murine leukemia virus for production of infectious progeny MSV. The presence of excess leukemia virus in stocks of MSV was directly demonstrated by production of complement-fixing leukemia virus antigen in mouse cultures infected with dilutions of the virus stocks beyond the focus-inducing end point (*4*). Thus stocks of MSV were shown to contain both sarcomagenic and leukemogenic virions.

Hamster tumors induced by MSV do not contain detectable infectious MSV. However, fully infectious MSV can be recovered by inoculating the hamster tumor cells either into intact mice or onto mouse embryo cultures that are infected with a murine leukemia virus (*3*).

The murine sarcoma-leukemia virus complex thus appears analogous to the avian Rous sarcoma (defective strain)–leukosis virus complex in its mixed virion composition, its ability to induce tumors in heterologous hosts, and in the recoverability of the virus from such heterologous tumors by similar procedures (*6*). Studies of the murine sarcoma virus appear potentially important in the comparative virology of sarcomagenic viruses in different species and as models for the possible recovery of the components of a human leukemia-sarcomagenic viral complex.

Hartley and Rowe (*4*) have pointed to two differences between the murine and avian viral complexes. The formation of the focal lesion produced by MSV in mouse cells requires the release of progeny infectious MSV, whereas the focus produced by Rous sarcoma virus (RSV) on chicken cells apparently can arise solely from cellular divisions originating in the initially transformed cell and in the absence of release of infectious virus. Also, the murine viral complex has a very high ratio of leukemia virion to sarcoma virion (up to 1000/1), whereas in the avian viral complex the leukosis virion/sarcoma virion ratio is much lower (10/1).

Inability of the MSV-induced focus to arise by cellular division alone has been confirmed in this laboratory (*7*). We have obtained evidence of some degree of viral defectiveness in focus assay on Swiss mouse embryo cells of a variety of stocks of murine sarcoma virus, obtained from either virus-induced mouse tumors or infected mouse cultures. The criterion of defectiveness employed was the apparent decrease in projected viral titer (number of foci × dilution factor) as judged by focus production on plating serial dilutions of the virus on mouse indicator cells (that is, "deviant" titration) and the increase in projected sarcoma virus titer, that