Isozymes in *Drosophila* cell line

P.K. Chakrabartty[1]

Genetics Department, University of Wisconsin-Madison, Madison (Wisconsin 53706, USA), 25 August 1977

Summary. Alcohol dehydrogenase, octanol dehydrogenase and fumarase isozyme patterns in *Drosophila* tissue culture cells were compared with the respective isozyme development pattern in the parental strain. The cell line lacks ADH activity and its fumarase isozyme pattern resembles the pupae and adult type.

Studies on the mechanisms of cell differentiation have been aided with the use of specific developmental markers[2]. In whole animal studies with *Drosophila* isozymes of esterases[3], phosphatases[4] have been used for this purpose. *Drosophila* embryonic tissue growth in vitro offers a homogeneous starting material and thus has expanded the possibilities for experimentation related to cell differentiation.

Drosophila cell line-1[5] has been characterized by the base composition and heterogeneity of its DNA[6]. The DNA contains a high amount of poly-dAT sequences characteristic of embryonic cells[7]. In the present study alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), and fumarase isozyme patterns of cell line-1 were compared with those of the ontogenic developmental stages of its parental strain. The cell line was found to lack any discernible ADH activity.

Materials and methods. Late embryonic tissue culture of *D. melanogaster* (cell line-1) kindly provided by Dr I. Schneider and maintained in our laboratory in Schneider's medium (GIBCO)[5] supplemented with 15% inactivated fetal calf serum. As a reference embryos, larvae, pupae, as well as adults of laboratory stock of *D. melanogaster* (strain Oregon R) were used to perform agar gel electrophoresis[8,9]. In 1 experiment ADH and fumarase activities were assayed spectrophotometrically as described[10,11].

Results and discussion. ADH activity of *Drosophila* varies greatly along the time axis of development[10]. Using spectrophotometric assay of ADH no measurable quantity of ADH was detected in the cell line extract. On agar gel electrophoresis 2 bands of ADH isozymes were visible in the fly homogenates (unpublished, also figures 1 and 2). No corresponding ADH band was visible in the cell line extract.

ODH patterns of monomorphic *Drosophila* species have been described[9]. Figure 1 shows the zymograms of *Drosophila* ODH isozymes during ontogenic development and of the tissue culture cells. It is evident that all of them share the same ODH pattern and are indistinguishable by this criterion. The 2 most cathodically migrating bands are that of ADH which uses Tris as substrate. The cell line extract lacks these bands.

3 bands of fumarase isozymes have been described for *Drosophila*[11]. Figure 2 shows that the most slow moving band is the most prominent isozyme of fumarase present in the embryos. Despite its origin from embryos the cell line does not share this property. It shares the fumarase isozyme phenotype with the pupae as well as adult.

Gradual decrease in the level of ADH in the primary culture of *Drosophila* was noted by Fox et al.[12]. In their system the cells would markedly respond to the addition or removal of ethanol by showing increased or decreased level of ADH. Thus ADH could comprize a system of substrate induced enzyme synthesis. In the present study, lack of ADH activity in the established cell line seems to be its response towards its chemical environment due to long-term culture in the absence of a supply of alcohol. Throughout the life cycle of *Drosophila* similar ODH zymograms are obtained but embryonic and postembryonic stages could be distinguished by fumarase patterns. Despite the embryonic type of genome characteristic and absence of ADH activity it seems that the cell line studied expresses some degree of differentiated function.

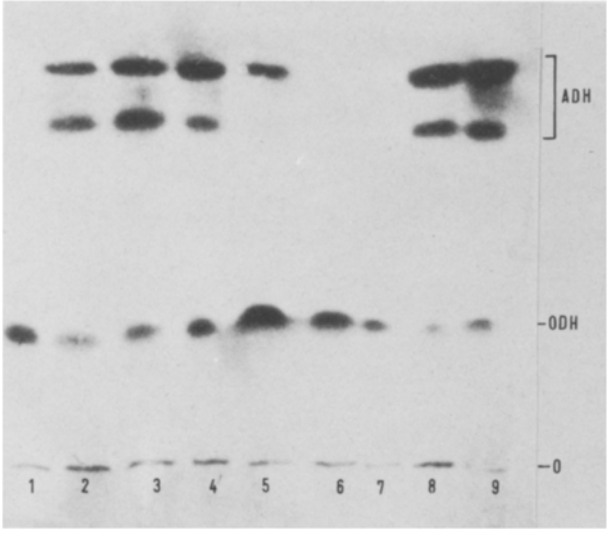

Fig. 1. ODH isozyme patterns in *Drosophila* and a cell line of *Drosophila:* (1,6,7) cell line-1, (3,9) pupae, (2,8) 3rd instar larvae, (5) early embryo, and (4) adult. 0, point of sample load. The most cathodically bands are ADH.

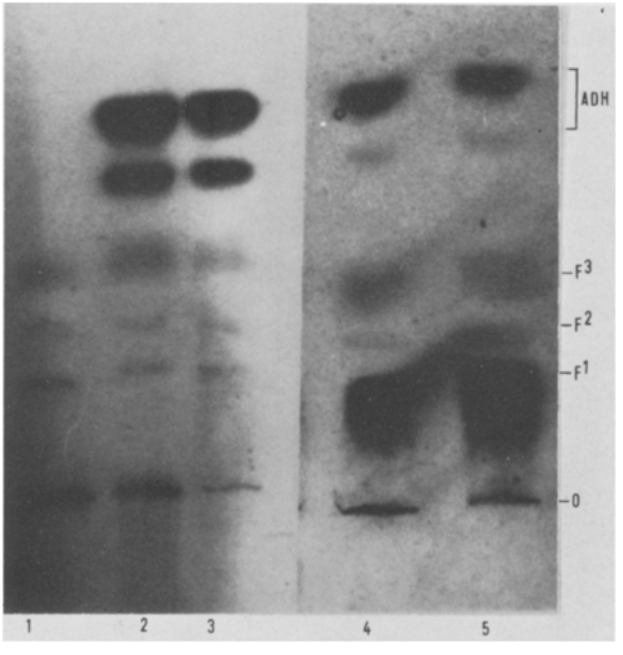

Fig. 2. Fumarase isozyme patterns in *Drosophila* and a cell line of *Drosophila:* (1) cell line-1, (2) pupae, (3) adult, (4) 1st instar larvae, and (5) embryo. F^1, F^2, and F^3 are the relative positions of 3 bands of fumarase isozymes. 0, point of sample load.

1 Present address: Laboratory of Pathophysiology, National Institutes of Health, Bethesda, Md. 20014, USA.
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The effect of ligand on the kinetics of the reaction of the sulfhydryl groups of human hemoglobin with p-mercuribenzoate

D.L. Currell, D. Young and J. Olea

Department of Chemistry, California State University, Los Angeles (California 90032, USA), 18 August 1977

Summary. The rate of reaction of PMB with the masked sulfhydryls of human hemoglobin derivatives correlates well with the extent of dissociation for various ligands. It is suggested that the $\alpha_1\beta_2$ dimer of hemoglobin participates in the slow step of the reaction with PMB.

In previous studies we have used the rate of reaction of the masked sulfhydryls with p-mercuribenzoate (PMB) as a probe of the extent of dissociation and/or conformation change of the protein under various conditions[1-3]. We have now extended these studies to include the effect of ligand on the kinetics of the reaction.

Materials and methods. Human hemoglobin was prepared by the toluene method[4]. After passage through an anion exchange resin the solution was dialyzed against phosphate buffer, pH 7. Methemoglobin was prepared by addition of $K_3Fe(CN)_6$ to oxyhemoglobin[5]. Fluoro- and azidomethemoglobin were prepared by addition of the sodium salt of ligand to methemoglobin[6]. A final concentration of 0.3 M NaF was necessary to convert about 95% of the methemoglobin into fluoromethemoglobin. Since this change in ionic strength would affect the hemoglobin dissociation, an equivalent concentration of NaF was added to the azidomethemoglobin for comparison. That the fluoride anion did not replace the azide ion was confirmed by comparing the visible absorption spectrum of the azidomethemoglobin with and without 0.3 M NaF. The spectra were identical. Experiments involving deoxyhemoglobin were carried out using a Thunberg type cell (Bolab, Inc., Derry, N.H.) which allowed addition of PMB under anaerobic conditions. The concentration of oxyhemoglobin was calculated from $E = 14.6 \times 10^3\ M^{-1}\ cm^{-1}$ (heme) at 575 nm and the concentration of methemoglobin from $E = 4.1 \times 10^3\ M^{-1}\ cm^{-1}$ (heme) at 630 nm. PMB was obtained from Calbiochem, La Jolla, California, and used without further purification. The concentration of PMB was calculated from $E = 1.69 \times 10^4\ cm^{-1}\ M^{-1}$ at 232 nm. The reaction rate of the masked -SH groups with PMB was measured spectroscopically at 25°±0.2 by a method described previously[1]. All spectrophotometric measurements were carried out with the Cary 14 spectrophotometer.

Results. The kinetic data were treated according to the method of Yuthavong and Ruenwongsa[7] instead of the pseudo 1st order plot previously used[1]. This method which uses only the initial rate was especially suitable for the reaction with methemoglobin which occasionally showed turbidity after 35 min reaction with PMB. Our results confirmed the rate equation found by Yuthavong and Ruenwongsa:

$$\text{Rate} = k_{obs.}\,[Hb]^{1/2}[PMB]$$

where [Hb] is the total hemoglobin concentration in heme and [PMB] is the concentration of PMB remaining after reaction of the β-93 sulfhydryls. The observed changes in rate with change in ligand are reported in the table.

Discussion. On the assumption that dissociation of the hemoglobin tetramer necessarily precedes the reaction of the masked sulfhydryls with PMB it is worthwhile to compare the observed rate of reaction with the extent of dissociation. The unreactivity of deoxyhemoglobin is understandable since the extent of dissociation is known to be significantly less than that of oxyhemoglobin[8]. It is noteworthy that α- and β-chains completely reacted with PMB exist largely as the tetramer below pH 7 in the deoxy-state[9]. The reactivity of azidomethemoglobin is consistent with the extent of its dissociation which at pH 6 is slightly less than that of methemoglobin[10] and approximately equivalent to that of oxyhemoglobin[8].

The apparently greater reactivity of fluoromethemoglobin is probably due to the presence of 0.3 M NaF which would promote further dissociation since its extent of dissociation is intrinsically somewhat less than that of the other methemoglobin derivatives, although the tertiary and quaternary structures of met- and fluorohemoglobin are identical[10,11]. This was demonstrated by the roughly equivalent rates of the fluoro- and azidoderivatives when 0.3 M NaF was added to the latter. The faster rate for methemoglobin in which the ligand is water is consistent with its greater dissociation as measured by several methods[10,12].

The observed rate expression provides evidence as to the identity of the reactive unit of hemoglobin participating in the slow step of the reaction. It has been shown that reaction of the β-93 sulfhydryls with PMB in the presence

Rate of reaction of masked sulphydryl groups of human hemoglobin derivatives with PMB in phosphate, pH 6.9, temperature 25 °C, NaCl = 0.1 M*

Derivative	$k_{obs.}$ ($M^{-1/2}\ min^{-1}$)
Deoxy-	No reaction
Oxy-	0.56
AzidoMet	0.51
AzidoMet + 0.3 M NaF	0.83
FluoroMet	0.78
Met-	1.27

* Hb concentration = $0.5\text{–}1.6 \times 10^{-5}$ M (Tetramer), PMB concentration = 1.1×10^{-4} M.