PURIFICATION AND PROPERTIES OF WILD-TYPE AND MUTANT GLUCOSE 6-PHOSPHATE DEHYDROGENASES AND OF 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM DROSOPHILA MELANOGASTER

SAMUEL H. HORI AND SOICHI TANDA

Department of Zoology, Faculty of Science, Hokkaido University, Sapporo

Received February 18, 1980

Glucose 6-phosphate dehydrogenase was purified to a homogeneous state from Drosophila melanogaster imagoes made homozygous for the X chromosome of a mutant male which showed two bands of enzyme activity on polyacrylamide gels as well as from Oregon R flies similarly made homozygous which showed only one major band, and their properties were compared with respect to molecular weight, pH optimum, specific activity, Km values and sensitivity to p-chloromercuribenzoate, MgCl₂, dehydroepiandrosterone, heat and anti-Oregon R glucose 6-phosphate dehydrogenase antibody.

The fast and slow bands of mutant enzyme had molecular weights of 115,000 and 283,000, respectively, while the wild-type enzyme had a molecular weight of 120,000. Treatment with sodium dodecyl sulfate cleaved the three enzymes into a subunit having a molecular weight of 69,000. This suggests that the slow and fast bands of the mutant enzyme represent tetramers and dimers of single polypeptides, respectively. Since the mobility of the fast mutant enzyme was the same as that of wild-type enzyme, it is inferred that the mutation resulted in a change of the quaternary structure of enzyme, without affecting its net charge in this particular instance. The mutant enzyme was more heat-stable than the wild-type enzyme, but they did not differ in other respects.

6-Phosphogluconate dehydrogenase was also purified to a homogeneous state from the wild and mutant flies. No difference was found between the two strains of flies with respect to several parameters used.

INTRODUCTION

During the course of an electrophoretic survey of glucose 6-phosphate dehydrogenase (G6PD) polymorphism in natural populations of Drosophila melanogaster we found a mutant which showed two bands of G6PD activity with some satellite bands
on polyacrylamide gels. Flies made homozygous for the mutant X chromosome as well as the F_1 females of the cross between wild and mutant strains also showed the two bands without exception, whereas the F_1 males showed maternal patterns. These observations strongly suggest that this mutant is similar to, if not the same as, the well-known G6PD mutant called B variant (O'Brien and MacIntyre 1969; Young et al. 1964). The difference between the single-band pattern of A variant and the double-band pattern of B variant has been explained in terms of the molecular instability of A variant by Steele et al. (1968), but we had impression that our mutant G6PD might be the enzyme which tends to form tetramers to a greater extent than to form dimers. In order to test this postulate, the mutant enzyme was purified to a homogeneous state and its properties were compared with those of wild strain flies, which showed only one major band. As a result, it was found that two bands of the mutant enzyme represent the dimer and tetramer of a single polypeptide.

6-Phosphogluconate dehydrogenase (6PGD), another oxidative enzyme of the pentose phosphate shunt, was purified from the two strains of flies, and their properties were compared in order to see if the mutant strain also possesses an altered 6PGD.

**MATERIALS AND METHODS**

*Drosophila melanogaster* were collected in Sapporo Brewery Co. in August, 1978. Wild males were individually crossed with females from a balanced stock (CIB/dor, f) which was supplied by Dr. T. K. Watanabe, the National Institute of Genetics, Japan and several isogenic strains were established (G6PD and 6PGD are both coded by the X-linked genes). Among these strains, a strain showing two bands of enzyme activity (B18) and a strain showing only one major band (B49) were used as materials in addition to a strain similarly made homozygous for the X chromosome of a male of the Oregon R stock which is characterized by a single, fast band. Flies were raised at 23°C on standard cornmeal-sugar-yeast-agar medium, and 1-14 day-old imagines were used for experiments.

**Electrophoresis:** Electrophoresis was carried out at 4°C on 7.5% polyacrylamide gels (Davis 1964) using a slab gel apparatus. Gel plates measured 0.1×14×10 cm with 12 sample slots, 1 cm width. Flies were individually homogenized using a small polyethylene homogenizer of Potter-Elvejhem type with 50 μl of 50 mM tris-HCl buffer, pH 8.5, containing 0.12 mM NADP, 7 mM 2-mercaptoethanol and 5 mM EDTA, and centrifuged at 10,000g for 10 min. The supernatant was mixed with an equal volume of 40% sucrose and applied on top of gels without using spacer and sample gels. In the case of 6PGD, glycerol was added to gels at a concentration of 25%. Electrode buffer was 0.077 M glycine-tris, pH 8.3. To cathode buffer were added 12 μM NADP and a drop of 0.1% bromophenol blue. A constant current of 5 mA/gel was applied until the dye travelled 3 cm. After electrophoresis, the gels were stained for G6PD or 6PGD activity at 37°C in the medium containing 50 mM tris-HCl buffer, pH 7.5, 0.6 mM G6P or 6PG, 0.06 mM NADP, 0.24 mM 3(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyl tetrazolium bromide and 33 μM phenazine methosulfate, or for protein with Amido black.
Electrophoresis was also performed on strips of Cellogel using 0.1 M tris-2 mM EDTA-borate buffer, pH 8.6. For studying the effects of pH and ionic strength of buffers on multiple molecular forms of G6PD, phosphate buffer and borate-NaOH buffer were also used (pH 7.0-9.0; I=0.05-0.15).

**Interconversion of multiple molecular forms:** Effects of dithiothreitol, cystamine HCl and trypsin on multiple molecular forms of G6PD were examined in the following manner: homogenates of B49 and B18 flies were prepared with tris-HCl buffer, pH 8.5, as described in the foregoing section in the presence of dithiothreitol (1-20 mM) or cystamine HCl (1-10 mM). The supernatant was treated with ammonium sulfate (35 g/100 ml) and the precipitate was dialyzed overnight against 20 mM phosphate buffer, pH 6.4, supplemented with either dithiothreitol or cystamine at the above concentrations. After dialysis, the enzyme solution was centrifuged and the supernatant was examined electrophoretically. For trypsin digestion, the 10,000g supernatant of fly homogenates was treated with varying amounts of trypsin (0.25-5 µg/fly) for 15 min at 20°C and immediately electrophoresed. For inhibiting proteolytic degradation of enzymes, flies were homogenized, treated with ammonium sulfate and dialyzed as described above in the presence of 0.4 mM phenylmethylsulfonyl fluoride (PMSF) or soybean trypsin inhibitor (25 µg/fly).

**Molecular weight estimation:** Molecular weight was estimated by the methods of Zwaan (1967) and of Weber and Osborn (1969). Standard proteins and their molecular weights were: ovalbumin, 45,000; bovine serum albumin, monomer, 67,000, dimer, 134,000; rat phosphorylase a, monomer, 97,000, dimer, 194,800; yeast isocitrate dehydrogenase, 300,000.

**Enzyme and protein assays:** Enzyme activity was assayed spectrophotometrically by following NADP reduction at 340 nm. The reaction mixture contained in a total volume of 1 ml: tris-HCl buffer (I=0.09; pH 7.5 for Km determination and for examination of effects of chemicals; pH 8.5 for other purposes), 6 mM G6P or 6PG and 0.6 mM NADP. One unit of activity was defined as the amount of the enzyme that reduced 1 µmol of NADP per min at 21°C. Effects of MgCl₂ (10 mM), p-chloromercuribenzoate (1 mM) and dehydroepiandrosterone (70 µM) were examined by incorporating them into the tris-HCl buffer prior to assay. Heat stability of enzyme was tested by incubating the purified enzyme in a water bath at 30-50°C for 5 min and at 30°C for 2 h. For examining effects of antibody on enzyme activity, a series of enzyme solution, 10 µl (about 5 milliunits) were mixed with 2-8 µl of antiserum (25-fold dilution) and the total volume was adjusted to 0.1 ml with a diluent (20 mM tris-HCl, pH 7.5 / 0.9% NaCl / 0.1% bovine serum albumin). After standing at 20°C for 1 h, the enzyme reaction was started by adding 0.9 ml of the substrate-buffer mixture. Protein was assayed by the method of Lowry et al. (1951).

**Immunological techniques:** Antibody to purified G6PD was raised in an albino rabbit by injecting 127, 80 and 90 units of enzyme in Freund's complete adjuvant on day 0, 21 and 28, respectively. Five days after the last injection, blood was collected and the antiserum was separated and stored at -70°C until use. Ouchterlony agar diffusion
was performed at room temperature in 1.5% agarose gel/25 mM tris-maleate buffer, pH 7.5/50 mM NaCl/0.5 mM EDTA.

**Purification of G6PD:** All purification procedures were carried out at 4°C with buffers containing 7 mM 2-mercaptoethanol and 1 mM EDTA. Flies, 100 g, were homogenized with 10 volumes of 20 mM phosphate buffer, pH 6.4/12 μM NADP/0.2 mM PMSF and centrifuged (10,000 g, 10 min). After adjusting pH to 7.2 with 2 M tris, the supernatant was treated with ammonium sulfate (25 g/100 ml) and the precipitate was removed by centrifugation. The soluble fraction was again treated with ammonium sulfate (10 g/100 ml of original supernatant) and the precipitate was dialyzed against 2 l of 20 mM phosphate buffer, pH 6.0/6 μM NADP/0.1 mM PMSF with three changes. The dialyzed solution was then centrifuged and the supernatant (50 ml) was placed on a CM-Sepha-
dex column (100×22 mm, i.d.) equilibrated with the dialysis buffer. The column was washed with 500 ml of 20 mM phosphate buffer, pH 6.4/6 μM NADP at a flow rate of 240 ml/h. The enzyme was eluted with 150 ml of 20 mM phosphate buffer, pH 6.9, at a flow rate of 120 ml/h. The peak fractions (85 ml) were pooled and placed on a DEAE-Sephadex column (30×20 mm, i.d.) equilibrated with 20 mM phosphate buffer, pH 6.4 and the column was washed with 100 ml of 20 mM phosphate buffer, pH 6.0, at a flow rate of 120 ml/h. The enzyme was eluted with 40 ml of 0.15 M KCl in the buffer, and the peak fractions (30 ml) were diluted with 1/2 volume of 20 mM phosphate buffer, pH 6.0. After adjusting pH to 6.2, the enzyme was placed on a 2', 5'-ADP Sepharose 4B column (30×10 mm, i.d.) equilibrated with 20 mM phosphate buffer/0.1 M KCl, pH 6.2. The column was washed first with 30 ml of 30 mM sodium pyrophosphate in 20 mM phosphate buffer, pH 6.6 and then with 100 ml of 0.1 M phosphate/0.1 M KCl, pH 6.6. The enzyme was eluted with 0.24 mM NADP in 0.1 M phosphate buffer, pH 8.0. The flow rate was 60 ml/h.

**Purification of 6PGD:** The supernatant obtained after precipitation of G6PD with ammonium sulfate (35 g/100 ml) was used as a starting material. It was treated with ammonium sulfate (10 g/100 ml of original supernatant) and the precipitate was dialyzed against 20 mM phosphate buffer, pH 6.0, containing 6 μM NADP. The dialyzed solution (20 ml) was placed on a CM-Sephadex column (70×32 mm, i.d.) and the gel was washed with 400 ml of the buffer. The enzyme was then eluted with 100 ml of 20 mM phosphate buffer, pH 6.6 and fractions of high enzyme activity were pooled, and adjusted to pH 6.2 with 0.1 M acetic acid after addition of 1/9 volume of 1 M KCl. The solution (50 ml) was then applied on a 2', 5'-ADP Sepharose 4B column (70×10 mm, i.d.) prepared as above. After washing with 100 ml of 0.2 M KCl in 20 mM phosphate buffer, pH 6.4, the enzyme was eluted with 40 ml of 0.1 M sodium citrate in 10 mM phosphate buffer, pH 6.4. Fractions of high enzyme activity were pooled and concentrated in an Amicon concentrator equipped with a PM 10 membrane.
RESULTS

Electrophoretic patterns of G6PD

Homogenates of B49 and Oregon R strains exhibited one major and one or two minor bands of G6PD activity on polyacrylamide gels (Fig. 1). A similar pattern was also observed on Cellogel, though their relative mobility was not the same as on polyacrylamide gels. These bands are designated F₁, F₂ and F₃ in the increasing order of anodal mobility, respectively. On the other hand, B18 flies showed a slow major band (S₁) and its satellite band (S₂) in addition to a fast moderately stained band (SF₁) accompanied by a faint band (SF₂). The SF₁ moved as fast as the F₁.

Electrophoretically distinguishable microheterogeneity has been reported with rat G6PD; i.e., the rat G6PD exists as three subforms named F₁, F₂ and F₃ (Hori and Yonezawa 1972) which are interconvertible with each other in vitro in the presence or absence of mercaptans. Since such microheterogeneity of rat G6PD resembles that of Drosophila G6PD, we tested if the Drosophila G6PD subforms are also interconvertible. For this purpose, homogenates of B49 flies were prepared in the presence of dithiothreitol or cystamine HCl, treated with ammonium sulfate, dialyzed, and subjected to electrophoresis, but the results showed no interconversion of F subforms. It was found, however, that if the dialyzed solution was allowed to stand at 4°C for more than 2 days, the F₁ and F₂ were converted into the F₃, irrespective of whether the sample was treated or untreated with dithiothreitol or cystamine HCl. This implies a

Fig. 1. Polyacrylamide gel electrophoresis of Drosophila G6PD. A and B, homogenates of B49 flies before and after 2 days of storage at 4°C, respectively. Note that the enzyme is converted into the F₃ form during storage. C and D, homogenates of B49 flies treated with trypsin (5 μg/fly) and phenylmethylsulfonylfluoride (PMSF, 0.4 mM), respectively. The trypsinsized sample was electrophoresed after 15 min, whereas the PMSF-treated sample was stored for 2 days at 4°C before electrophoresis. Note that trypsin induces the conversion of the F₁ and F₂ into the F₃, while PMSF-treatment prevents the conversion occurred during storage. E and F, homogenates of B18 flies before and after treatment with trypsin, respectively. Note that the S₁ and SF₁ are converted into the S₂ and SF₂, respectively, by trypsin digestion. The SF₁ moves as fast as the F₁.
proteolytic modification of G6PD during ageing. To test this possibility, the 10,000 g supernatant of fly homogenates was treated with varying amounts of trypsin for 15 min at 20°C. Electrophoresis of the treated samples clearly demonstrated that the F₁ is converted into the F₂ at a trypsin concentration of 0.5-1.0 µg/fly and into the F₃ at 5 µg/fly. In addition, the conversion during ageing was found to be prevented if fly homogenates were treated with 0.4 mM phenylmethylsulfonylfluoride or trypsin inhibitor.

A series of similar experiments with B18 strain also indicated that the S₁ and SF₁ can be converted into S₂ and SF₂, respectively, by a mild trypsin digestion, and that the conversion during ageing is inhibited by proteinase inhibitors (Fig. 1). These findings clearly indicate that the F₂, F₃, S₂ and SF₂ are hydrolytic products of the F₁ and S₁. Since fresh fly homogenates show one or more modified forms even when prepared with proteinase inhibitors, such subforms might exist also in vivo.

In the above experiments using polyacrylamide gels, conversion of S₁ into SF₁ and vice versa was never observed. Likewise, no conversion was induced on Cellogel even if the standard tris-EDTA-borate buffer was replaced by phosphate buffer or borate-NaOH buffer at various pH and at various ionic strength.

Properties of G6PD

The results of a typical purification experiment with Oregon R flies are given in Table 1. The purified enzyme was homogeneous electrophoretically and immunologically (Fig. 2).

The properties of G6PD purified from Oregon R and B18 strains are given in Table 2. It is clear that the two enzymes do not differ with respect to the parameters shown. The purified B18 enzyme exhibited the same double band pattern as the fly homogenates, and the molecular weight of the two bands were estimated to be 115,000 and 283,000, respectively (Fig. 3). On the other hand, the enzyme showed only one band on SDS-polyacrylamide gels, whose molecular weight was about 69,000 (Fig. 4). Although the value for the fast band was consistently lower than expected for some reason, these results strongly suggest that the two bands observed on SDS-free gels would represent dimers and tetramers of single polypeptides. The molecular weight of Oregon R G6PD subunit was also 69,000, and that of the dimer was 120,000 (Figs. 3 and 4).

Table 1. Purification of Drosophila melanogaster G6PD

<table>
<thead>
<tr>
<th>Step</th>
<th>Purity (mg)</th>
<th>Total enzyme activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant*</td>
<td>3,827</td>
<td>421</td>
<td>0.11</td>
<td>100</td>
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<td>Ammonium sulfate</td>
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<td>283</td>
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<td>CM-Sephadex</td>
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<td>194</td>
<td>11.9</td>
<td>46.1</td>
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<tr>
<td>DEAE-Sephadex</td>
<td>3.1</td>
<td>170</td>
<td>54.8</td>
<td>40.4</td>
</tr>
<tr>
<td>2', 5'-ADP-Sepharose</td>
<td>0.64</td>
<td>127</td>
<td>198</td>
<td>30.2</td>
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</tbody>
</table>

* 10,000 g supernatant from 100 g flies.
Figure 5 shows the loss of enzyme activity after 5 min heating at various temperatures. The B18 enzyme appears to be more resistant to heat at 37-50°C than Oregon R enzyme. However, no loss of activity was observed at 30°C even after 2 hours of heating with both enzymes. This implies that the mutation did confer heat stability on the enzyme, but only to a physiologically insignificant extent.

Figure 6 illustrates neutralization curves obtained when increasing amounts of anti-Oregon R G6PD antiserum were added to given amounts of G6PD from the two strains. The relative inhibition potencies (slope of the straight portion of the curve) did not

<table>
<thead>
<tr>
<th>Specific activity (units/mg)</th>
<th>Km (μM, pH 7.5)</th>
<th>pH optimum</th>
<th>Activity* in the presence of 10 mM MgCl₂</th>
<th>1 mM PCMB**</th>
<th>70 μM DEA***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G6P or 6PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oregon R</td>
<td>198</td>
<td>63</td>
<td>12</td>
<td>8.5</td>
<td>109</td>
</tr>
<tr>
<td>B 18</td>
<td>199</td>
<td>63</td>
<td>11</td>
<td>8.5</td>
<td>104</td>
</tr>
<tr>
<td>Oregon R</td>
<td>42</td>
<td>53</td>
<td>47</td>
<td>8.5</td>
<td>113</td>
</tr>
<tr>
<td>B 18</td>
<td>42</td>
<td>57</td>
<td>42</td>
<td>8.5</td>
<td>112</td>
</tr>
</tbody>
</table>

* Percentage of control.
** p-Chloromercuribenzoate.
*** Dehydroepiandrosterone.

Figure 5 shows the loss of enzyme activity after 5 min heating at various temperatures. The B18 enzyme appears to be more resistant to heat at 37-50°C than Oregon R enzyme. However, no loss of activity was observed at 30°C even after 2 hours of heating with both enzymes. This implies that the mutation did confer heat stability on the enzyme, but only to a physiologically insignificant extent.

Figure 6 illustrates neutralization curves obtained when increasing amounts of anti-Oregon R G6PD antiserum were added to given amounts of G6PD from the two strains. The relative inhibition potencies (slope of the straight portion of the curve) did not
differ significantly. In addition, the antiserum produced a single connecting precipitin line of G6PD activity without spurs against the two enzymes on agar plates. Thus, the two enzymes do not appear to be immunologically diverse.

**Properties of 6PGD**

The results of a typical purification experiment with Oregon R flies are given in Table 3. The purified enzyme was homogeneous electrophoretically (Fig. 7). The properties of 6PGD from Oregon R and B18 strains are given in Table 2. It is clear that the two enzymes do not differ significantly. The molecular weights of the two enzymes were the same, being 105,000 on SDS-free gels and 58,000 on SDS-gels. It was thus concluded that 6PGD exists as homodimers in the two strains.

**Mating experiments**

The results of single pair matings of B18 and B49 flies (Fig. 8) unequivocally demonstrate an X-linked transmittance of the two codominant alleles of G6PD gene.

**DISCUSSION**

The methods for purifying G6PD and 6PGD from *D. melanogaster* to a homogeneous state were described for the first time in this report.
Fig. 4. Molecular weight estimation by means of SDS-polyacrylamide gel electrophoresis. 1, ovalbumin; 2, bovine serum albumin; 3, rat phosphorylase a (skeletal muscle); 4, Oregon R 6PGD; 5, Oregon R G6PD. B18 6PGD and G6PD migrate as fast as Oregon R 6PGD and G6PD, respectively. Ordinate, relative mobility.

Fig. 5. Heat inactivation test. G6PD was heated at 30-50°C for 5 min and assayed.

Fig. 6. Enzyme neutralization curves of G6PD from Oregon R and B18 strains with antiserum to Oregon R G6PD.
The properties of purified Drosophila G6PD are quite similar to those of other invertebrate G6PD so far examined in our laboratory, except that the Km value for G6P is considerably high in Drosophila as in hydra and earthworm (Matsuoka and Honi 1980; Ohnishi and Hori 1977). Although kinetic data on invertebrate 6PGD are not available, Drosophila 6PGD appears to be peculiar in that the Km for NADP is rather high (about 40 pM) when compared with rat 6PGD (13 pM, Procatal and Holten 1972).

Two electrophoretic variants of G6PD have been known to occur in wild-type strains of D. melanogaster (Young et al. 1964). One of the variants designated as A is a single, fast moving band, while the other, B variant is a double band consisting of an intense, slow band and a faint, fast band. The latter migrates as fast as the A variant. Various combinations of single pair matings provided evidence that there are two codominant alleles transmitted by way of the X chromosomes. Experiments with two stocks of attached-X females favored this view (Young et al. 1964). However, unlike

<table>
<thead>
<tr>
<th>Protein (mg)</th>
<th>Total enzyme activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant*</td>
<td>913</td>
<td>73</td>
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<td>Ammonium sulfate</td>
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<tr>
<td>CM-Sephadex</td>
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<td>1.59</td>
</tr>
<tr>
<td>2', 5'-ADP-Sepharose</td>
<td>0.36</td>
<td>13</td>
<td>36.1</td>
</tr>
</tbody>
</table>

* 10,000 g supernatant from 30 g flies.

![Image of SDS-polyacrylamide gel electrophoresis](image_url)

**Fig. 7.** SDS-polyacrylamide gel electrophoresis of 6PGD purified from B18 and Oregon R flies (22 µg enzyme per tube). Gels were halved after electrophoresis and stained with Amido black.
many other enzymes, heterozygous females showed a double band pattern without an intermediate band. This phenomenon was extensively studied by Steele et al. (1968). Their conclusion was that the absence of an intermediate band in heterozygous females is due to the absence of charge difference between the two variants, that the existence of a hybrid enzyme can be manifested on electrophoretic gels by taking advantage of difference in heat stability of the variants and that the difference in mobility between the two variants may be ascribed to molecular instability of the A variant.

The mutant G6PD reported in this paper has characteristics quite similar to the B variant reported by Young et al. (1964) and Steele et al. (1968); i.e., both are characterized by a double band pattern in homo, hemi- and heterozygous individuals, a large molecular weight which is about two-fold that of normal enzyme, and heat stability. The only difference between the B and the present variants is concerned with the Km values for G6P and NADP; in the B variant, the values are 170 μM and 58 μM in contrast to 53 μM and 11 μM in our variant. However, Steele et al. (1968) regarded the values not as significantly different from those of wild-type enzyme, as we did with our enzymes. Accordingly, it might be possible that our mutant would be the same as or very similar to the B variant.

The anomalous electrophoretic behavior (a double band pattern) of our mutant G6PD, and probably also that of the B variant, may be fully explained if one assumes that the mutant G6PD exists as tetramers as well as dimers of single polypeptides which have the same or nearly the same net charge as the wild-type polypeptide, and that this wild-type polypeptide exists only as dimers.

Our survey on animal G6PDs has revealed that the quaternary structure of G6PD differs from species to species; some species, such as mammals, reptiles and aves possess dimers in excess of tetramers, whereas fishes, amphibians and some invertebrates
possess tetramers in excess of dimers (Hori and Yonezawa 1972; Sado and Hori 1976; Ohnishi and Hori 1977). Thus, there appears to exist an equilibrium between dimers and tetramers of this enzyme that shifts in favor of dimers in some species and in favor of tetramers in other species depending upon the primary structure of the monomer. In fact, conversion of tetramers into dimers can be manipulated *in vitro* in some species (rat, crayfish, earthworm and hydra; Hori and Yonezawa 1972; Ohnishi and Hori 1977). If this holds, it is not unreasonable to assume that a single or a few point mutations would cause a shift of the equilibrium to the direction opposite to that in wild-types. This would probably be the case found in Drosophila.

Komma (1966) reported that both sexes of the B variant have significantly more activity than the A variant, whereas Steele *et al.* (1969) found a high activity only in males of the B variant. Our preliminary data also showed that G6PD activity is higher in both sexes of our mutant than in Oregon R strain. However, this activity difference cannot be attributed to the difference in catalytic properties, since both mutant and wild-type enzymes have the same specific activity as shown in Table 2. The higher activity in the mutant might, therefore, be caused by an increased rate of enzyme synthesis or by a decreased rate of enzyme degradation or both, or by still other mechanisms. This is currently under study.

Giesel (1976) described Zw\(^A\) and Zw\(^B\) as two non-allelic, autosomal genes which segregated for three and two alleles, respectively, and claimed that whether Zw\(^A\) or Zw\(^B\) or both show activity depends on the genotype of a sex-linked gene, in striking contrast to the opinion of Young *et al.* (1964). It is impossible at present to afford a conclusive proof or disproof of the validity of Giesel's opinion, but it seems that Giesel's multiple alleles might probably be due to misinterpretation of the zymogram where some post-translational modifications were regarded as allozymes, judging from Fig. 1 of his paper. The number and relative mobility of Giesel's allozymes coincide well with those of proteolytic degradation products of intact enzymes reported in this paper.

**ACKNOWLEDGMENT**

We wish to thank Dr. M. T. Kimura and Mr. M. Iwata for their help in the establishment of isogenic strains of flies. This work was supported in part by a grant-in-aid Scientific Research from the Ministry of Education.

**Note added in proof**

After submitting this paper we found that the Km value for NADP significantly differs between crude and purified 6PGD, being 12 and 40 \(\mu\)M, respectively. The cause of such difference is now being studied.
LITERATURE CITED


