molecular weight 500,000 and sedimentation coefficient 18*S* (*7*) is calculated as 120 Å. This value is in excellent agreement with the measured dimensions of the cubical particles (120 Å edge) as shown by electron microscopy using several preparation techniques.

Subunits in the model proposed are not related by the principle of quasi-equivalence that governs the structure of many small viruses (*10*). In the model proposed, subunits are found in three positions: on faces, edges, or vertices. We assume that the plane net, from which the structure may formally originate, consists of a square lattice. Then the distortion required to place a subunit on a vertex, maintaining the same bonding rule, becomes unacceptable (*10*). We are therefore obliged to propose either that subunits are capable of making several kinds of bond, or that subunits undergo considerable conformational changes as a result of their statistical location in the completed particle.

From the foregoing, fraction I protein consists of a cube, about 120 Å along each edge, containing 24 subunits as seen in high-resolution electron micrographs. The protein contains all the carboxydismutase activity recoverable from glycerol gradients. It has the same sedimentation coefficient and molecular weight as bacterial RNA-polymerase, but lacks the latter's activity. The particle structure does not appear to be governed by the same rules that apply in the case of protein shells of small viruses. We believe this to be the first occasion upon which the substructure of a protein enzyme of about 120 Å diameter has been resolved in electron micrographs.

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X-Linked 6-Phosphogluconate Dehydrogenase in Drosophila: Subunit Associations

Abstract. *6-Phosphogluconate dehydrogenase (PGD) subunits have been dissociated and reassociated in vitro. The intermediate PGD of the heterozygous phenotype is reproduced by reassociation in vitro of subunits derived from the two types of PGD from homozygous flies. This result has a bearing on gene action in the diploid organism and on the mechanism of dosage compensation in* Drosophila melanogaster.

Dosage compensation in mammals is probably accomplished by inactivation of one X chromosome in each female somatic cell (*1*). In the female, when one allele of an X-linked gene determines the structure of the wild-type protein and the other allele produces a mutant protein, each cell contains either the wild type or the mutant type of protein, but not both. Although dosage compensation has been repeatedly demonstrated in *Drosophila*, a specific mechanism is unknown (*2*). Evidence from position-effect variegation at the white locus suggests that both X chromosomes are active in female cells (*2*). We now report that both alleles of the X-linked gene, which determines the structure of 6-phosphogluconate dehydrogenase (PGD), function in those cells in which this enzyme is synthesized in the *D. melanogaster* female.

Young has located the PGD structural gene (*Pgd*) at approximately 0.9 on the X chromosome (*3*). Certain strains have a single PGD band whose electrophoretic mobility is greater than that of the single PGD band of other strains. These phenotypes were called PGD A and PGD B, respectively. The mating of a PGD A individual with a PGD B fly yielded a third phenotype in all female offspring, which was characterized by the PGD A and PGD B bands along with a more-densely staining PGD band that was precisely intermediate in mobility; the intermediate band was designated PGD I^{AB}.

The molecular configuration that best accounts for the single intermediate band in the heterozygous female is that of a dimer. In the cells of this fly two different subunits, designated s^a and s^b (*4*), could assemble to produce three dimers. PGD A, PGD B, and PGD I^{AB} would then be composed of s^as^a, s^bs^b, and s^as^b, respectively. Random combination of two different subunits would yield two molecules of s^as^b for every s^as^a or s^bs^b. It also follows that each allele of the *Pgd* gene would specify either an s^a or s^b subunit. Demonstration that the intermediate band is indeed composed of subunits contained in the PGD A and PGD B enzymes would require dissociation of the subunits of those enzymes and their reassociation to produce the PGD I^{AB}. Dissociation was accomplished by treatment of a partially purified PGD enzyme with propanedithiol, a compound that breaks disulfide bonds; reassociation occurred after dialysis and concentration of the dialyzate. All purification procedures were carried out at 4°C.

Twenty grams of adult flies (about 25,000) mixed with 3.6 g of Norit A were homogenized in 400 ml of 0.02*M* phosphate buffer, *p*H 6.2 and $10^{-4}M$ with respect to ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 18,000*g* for 75 minutes and the precipitate was discarded. Solid $(NH_4)_2SO_4$ was added to the supernatant to give a 46-percent $(NH_4)_2SO_4$ solution which was stirred for 30 minutes and centrifuged; the precipitate was discarded, and the supernatant was adjusted to 58 percent $(NH_4)_2SO_4$ by addition of the salt. After another 30 minutes of mixing, this precipitate, which contained

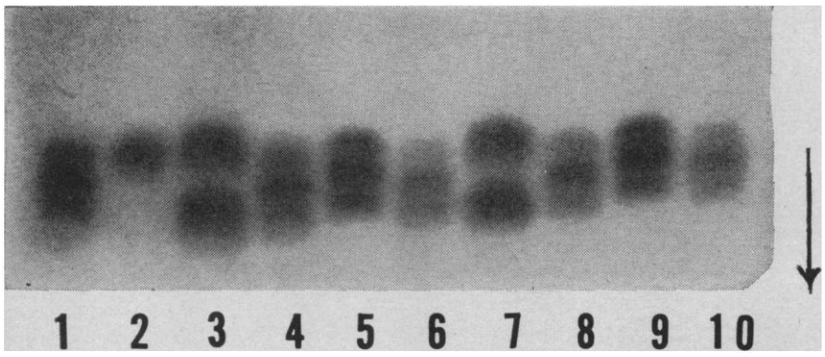

Fig. 1. Starch gel stained for PGD. Slots 3 and 7 are an untreated mixture of PGD A and PGD B. Slots 5 and 9 are an untreated heterozygous phenotype. Slot 2 is a PGD B treated with propanedithiol. Slots 1, 4, and 8 and slots 6 and 10 are two different mixtures of PGD A and PGD B treated with propanedithiol.

the enzyme, was collected by centrifugation and dissolved in 50 ml of a $10^{-4}M$ EDTA solution. This solution was then dialyzed for 2.5 hours against $0.005M$ acetate buffer, *p*H 5.2, containing $10^{-4}M$ EDTA. The precipitate that formed was removed by centrifugation and the supernatant was passed through a carboxymethylcellulose column that had been equilibrated with $0.005M$ phosphate buffer, *p*H 5.7, and $10^{-4}M$ with respect to EDTA. This eluate was then applied to a diethylaminoethylcellulose (DEAE-cellulose) column that had been packed with 1 g of cellulose per 15 mg of protein in the eluate and equilibrated with $0.005M$ phosphate buffer, *p*H 6.5 and $10^{-4}M$ with respect to EDTA. An elution with $0.02M$ NaCl in buffer was made, and the eluate was discarded. The enzyme was then eluted with $0.08M$ NaCl in buffer. The enzyme obtained from the DEAE-cellulose step had a specific activity of 2.5 to 3.0 units per milligram of protein and was stable for weeks in this solution at 4°C. (One enzyme unit is the quantity of enzyme that reduces 1 μmole of nicotinamide adenine dinucleotide phosphate per minute under the assay conditions employed.) PGD was assayed spectrophotometrically by the method of Horecker (*5*), with minor modifications; protein concentration was determined by the Lowry method (*6*).

Dissociation and reassociation of the PGD molecule was accomplished in the following manner. To 0.15 enzyme units per milliliter of the DEAE-cellulose eluate, propanedithiol was added to make a solution that was $6 \times 10^{-2}M$ as to propanedithiol; after incubation for 1 hour at 37°C, the solution was dialyzed against $0.005M$ phosphate buffer, *p*H 6.5, for 4 to 4.5 hours at 24°C. The resultant dialyzate was concentrated by ultrafiltration under partial vacuum, and the concentrate was subjected to starch-gel electrophoresis (*7*).

The results appear in Fig. 1. A mixture of the partially purified PGD A and PGD B demonstrates these two phenotypes (slots 3 and 7). The heterozygous phenotype with the more densely staining PGD I^{AB} band is also seen (slots 5 and 9). A PGD B sample treated with propanedithiol shows no change on starch gel from the control PGD B (slot 2); although it is not shown here, the starch-gel characteristics of PGD A also were unchanged by treatment with propanedithiol. Two mixtures of PGD A and PGD B that were treated with propanedithiol clearly demonstrate the more densely staining PGD I^{AB} band of the heterozygous phenotype (slots 1, 4, and 8 and slots 6 and 10).

Three bands have also been found in the heterozygous phenotype of PGD in man and the rat (*8*), the deer mouse (*9*), and the pigeon (*10*). Many other enzymes from heterozygous individuals demonstrate an intermediate band on starch gel (*9*). Schwartz first postulated from his work with a maize esterase that the fast, slow, and intermediate molecular forms represent dimers that are produced by combination of two different subunits (*11*). Certain dimeric enzymes that are produced by the associations of two different subunits have been conclusively demonstrated in some haploid individuals into which a second genome had been introduced by conjugation or by heterokaryon formation (*12*). However, the presence of an intermediate band in the heterozygous phenotype has been the only evidence of a dimeric enzyme whose structure is controlled by a single locus in a diploid organism.

The discovery that the intermediate PGD is produced from subunits of PGD A and PGD B in *D. melanogaster* is noteworthy on two further counts. First, it means that each representative of the *Pgd* gene determines the structure of a polypeptide subunit that by itself is nonfunctional; thus s^a or s^b becomes an active enzyme only upon association with other s^a or s^b molecules. Second, the location of the *Pgd* gene on the X chromosome leads to the following consequences. If only one allele were functioning in any single cell of the heterozygous female, then only s^a or s^b subunits would be synthesized in that cell; thus only PGD A or PGD B would be made in any one cell. No cell could synthesize the PGD I^{AB} unless both X-chromosome alleles were active. An alternative possibility of formation of PGD I^{AB} due to the association of subunits of which one had been transported from its cell of origin to another cell is unlikely. Thus, as previously suggested (*2*), if both representatives of X-linked genes are active in *D. melanogaster*, dosage compensation in this organism cannot be accomplished by the mechanism attributed to mammals.

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