ROLE OF THE PENTOSE PHOSPHATE PATHWAY IN METABOLISM OF *DROSOPHILA MELANOGASTER* ELUCIDATED BY MUTATIONS AFFECTING GLUCOSE 6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASES

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1. Introduction

The pentose phosphate cycle in animal cells is considered usually as a major source of NADPH and pentoses needed for nucleic acid biosynthesis [1,2]. The oxidative steps of the pentose phosphate cycle are operated by glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.43). The non-oxidative reactions provide reversible interconversions of pentoses and metabolites of the glycolytic pathway. Estimations of the individual role of the oxidative and non-oxidative reactions in the pentose phosphate shunt are based usually on quantitative measurements of the activities of corresponding enzymes in tissue extracts [3,4]. However, an immediate and separate evaluation of the role of oxidative and non-oxidative routes in a living cell encounters difficulties because of the absence of specific inhibitors of given enzymatic reactions. Another approach to the elucidation of the role of metabolic pathways is to obtain mutations that specifically shut off a given enzymatic step. In the present paper two classes of biochemical mutations in *Drosophila melanogaster* resulting in elimination or decrease of the activity of G6PD or 6PGD are described. The properties of these mutations, their interaction and their influence on the viability of individuals were investigated. Although the alteration or elimination of 6PGD has a lethal effect, the viability of flies carrying both types of mutations shows non-essential role of the pentose phosphate pathway in the biosynthesis of NADPH. It is suggested that anaerobic biosynthesis of pentoses and related metabolites of glycolysis is the main role of the pentose phosphate pathway.

2. Methods

Determination of the activities of G6PD and 6PGD and polyacrylamide gel electrophoresis of isozymes was performed essentially as described earlier [5].

3. Results and discussion

The *Pgd* locus coding for 6PGD in *D. melanogaster* is located on the X-chromosome [5–7]. Mutations affecting this locus were described earlier [6]. These mutations resulted in zero or decreased 6PGD activity and led to the death of individuals. A 25–100-fold decrease in viability of flies carrying mutations in the *Pgd* locus was observed. The period of development was also greatly lengthened in these individuals. The extent of the decrease in viability caused by mutation is expressed as a percentage of mutant individuals to normal ones enclosed in the same bottle. Activity of 6PGD was assayed in extracts prepared from the adult flies carrying the mutations. The data in table 1 show a positive correlation between the decrease in viability and the extent of reduction of 6PGD activity. Semi-lethal mutations (N 13 and N 50) caused a 2–4-fold decrease of 6PGD activity and about a 2–7-fold drop in viability. In extracts of surviving flies carrying practically lethal mutations (NN 35, 39, 45, 71) less

Table 1
Correlation between the activity of 6PGD and viability of flies

Flies	Activity 6PGD	G6PD	Viability
Normal	100	100	100
Mutants:			
13	40–67	90–100	13
50	25–50		50
35	< 3	90–100	3
39	< 2		1.5
45	< 2		
71	< 4	90–100	1

Activity (80–160 nmol NADPH/min/mg protein) and viability of normal flies being taken as 100 per cent.

than 2–4% of the normal 6PGD activity was detected. The viability of these flies was about 25–100 times lower than normal. Mutations resulting in a decreased or zero 6PGD activity had no effect on the level of G6PD activity. Thus the levels of the activities of these dehydrogenases are determined independently by the corresponding genes.

Consideration of the data obtained may lead to the conclusion that the oxidative route of the pentose phosphate pathway is indispensable for *Drosophila* metabolism. The following data however permit one to postulate an opposite suggestion. We have succeeded in obtaining the suppressor mutations capable of restoring the viability and fertility of flies carrying the lethal (N 71) mutation which eliminated the activity of 6PGD. These suppressor mutations denoted as *su_1Pgd*, *su_2Pgd* and *su_5Pgd* were obtained by ethyl methanesulfonate treatment of flies as has been described [6]. It was surprising that no 6PGD or G6PD activity was detected in the extracts of flies carrying both the lethal N 71 and one of these suppressors. Consequently it is impossible to explain the restoration of viability as a result of a reversion of the mutant *Pgd* gene to the normal state. It was shown that the suppressor mutations caused a sharp decrease in G6PD activity as compared to the level in the *Drosophila* stock carrying the lethal N 71 mutation. The K_M values of G6PD for glucose 6-phosphate and NADP were determined in crude extracts of flies. The data shown in table 2 demonstrate that the suppressor mutations caused approx.

Table 2
The K_M values for glucose 6-phosphate and NADP in extracts of mutant and normal flies.

Flies	K_M for G-6-P	K_M for NADP
Normal	9.0×10^{-5} M	7.4×10^{-5} M
Mutants:		
Su_1Pgd	2.6×10^{-3} M	14.9×10^{-5} M
su_2Pgd	3.6×10^{-3} M	23.7×10^{-5} M
su_5Pgd	5.9×10^{-3} M	33.0×10^{-5} M

30–60-fold and 2–7-fold decrease in the affinity of G6PD for substrate and NADP respectively. The data obtained suggest that in *Drosophila* the oxidative route of the pentose phosphate cycle plays no essential role in the supply of NADPH or pentoses.

Genetical experiments (in press) have demonstrated that the suppressor mutations affect the *Zw* locus on the X-chromosome at a point separate from the *Pgd* locus. Two allelic variants, Zw^A and Zw^B, coding for the electrophoretically fast and slow forms of G6PD respectively have been described [7]. Polyacrylamide gel electrophoresis showed that all three suppressor mutations resulted in increased electrophoretic mobility, no doubt due to amino acid replacement, of the mutant variants of G6PD as compared to the wild type slow B-isozyme. Fig.1 shows this for the *su_2Pgd* mutation.

Mutations in the *Zw* locus without *Pgd* mutations have no effect on the viability or fertility of flies. This result is in accord with data presented by Geer et al. [8], who described a non-lethal mutation in the *Zw* locus of *D. melanogaster*. Our results also show no adaptive changes in the G6PD activity due to the decreased level of 6PGD.

The lethal effect of the mutations in the *Pgd* locus indicates that partial or complete inhibition of the 6-phosphogluconate dehydrogenase reaction is harmful for *Drosophila*. This lethal effect, however, may be suppressed by a sharp decrease in the level of G6PD. There is clear evidence that the simultaneous blocking of both oxidative reactions in the pentose phosphate pathway, usually considered as major sources of NADPH in cells, has no noticeable effect on the viability of individuals. The bulk of NADPH may be supplied in *Drosophila* by the operation of other metabolic routes. Recently others have thrown

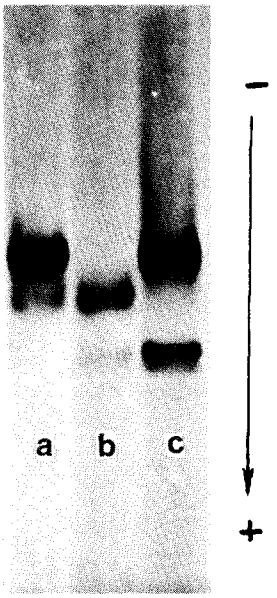

Fig.1. Electrophoretic mobility of the isozymes of G6PD. Mixtures of fly extracts: (a) B-isozyme (10 mg of protein) + +su_2Pgd (100 mg of protein); (b) A-isozyme (5 mg of protein) + su_2Pgd (100 mg of protein); (c) A and B isozymes (150 mg of protein of each extract).

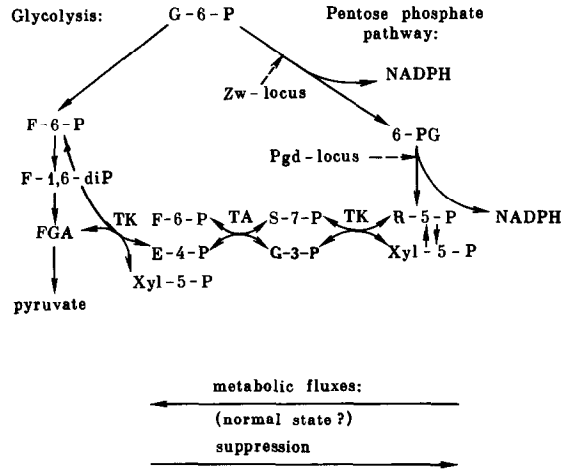

Fig.2. Metabolic relations between the pentose phosphate cycle and glycolysis. Abbreviations: G-6-P, glucose 6-phosphate; 6-P-G, 6-phosphogluconate; F-6-P, fructose-6-phosphate; F-1,6-diP, fructose 1,6-diphosphate; S-7-P, sedoheptulose 7-phosphate; R-5-P, ribose-5-phosphate; Xyl-5-P, Xylulose-5-phosphate; E-4-P, erythrose 4-phosphate; G-3-P, glyceraldehyde 3-phosphate; NADPH, reduced nicotinamide adenine dinucleotide-phosphate; TA, transaldolase; TK, transketolase.

in a note of caution on the widespread belief on an efficient working of G6PD and 6PGD in a NADPH producing system [9]. It would be an intriguing task to find the metabolic reactions that compensate for the deficit of NADPH due to "zero" mutations in the *Zw* and the *Pgd* loci.

The pentose phosphate cycle is also a potential source of pentoses. Pentose synthesis may be accomplished either by oxidation of glucose 6-phosphate or 6-phosphogluconate or by the reversible operation of transaldolase and transketolase using metabolites of glycolysis. For example in tumor cells the biosynthesis of pentoses from fructose 6-phosphate and glyceraldehyde 3-phosphate via the transketolase and transaldolase reactions occurs [4]. Fig.2 illustrates the relationship between the pentose phosphate cycle and glycolysis. The question arises whether the elimination of the metabolic disorder caused by a drop in G6PD activity could be explained by a restoration of pentose concentration to a normal level. Glucose 6-phosphate isomerase and G6PD may compete for glucose 6-phosphate as a substrate. The affinity of G6PD for glucose 6-phosphate is several times greater than that of the isomerase [4]. As a result, a substantial part of the glucose 6-phosphate pool may be oxidized by the pentose phosphate pathway. However the subsequent non-oxidative reactions may lead eventually to the synthesis of glyceraldehyde 3-phosphate and fructose 6-phosphate, intermediates in the glycolytic pathway. In the absence of 6PGD activity, this metabolic route is interrupted and metabolic equilibrium between glycolysis and the pentose phosphate cycle may be altered. Accumulation of 6-phosphogluconate may accentuate this deleterious effect, since high concentration of this metabolite may inhibit some steps in glycolysis, as was shown in mammals [10]. The drastic reduction of the activity of G6PD due to the mutation in the *Zw* locus may eliminate the metabolic disturbance by intensifying the flow of glucose 6-phosphate into glycolysis, with the consequent accumulation of intermediates able to produce pentoses via transaldolase and transketolase. In the normal state the metabolic flux may be directed from pentoses to

erythrose 4-phosphate, glyceraldehyde 3-phosphate and fructose 6-phosphate. Blocking of both the oxidative reactions may produce a viable situation, if reversal of metabolic flux from glycolysis to pentoses is achieved (fig.2.).

The data obtained are in a good accord with our observation that the development of *Drosophila* larvae carrying the "zero" *Pgd* mutation stops at the end of the first instar larval stage, at which time a severalfold increase of the activity of G6PD is attained [11].

The results discussed here permit one to conclude that the oxidative part of the pentose phosphate cycle may play no essential and indispensable role in the biosynthesis of NADPH. At any rate the supposed deficit of the NADPH concentration as a result of the elimination of the G6PD and 6PGD activities is readily compensated. A major vital role of the pentose phosphate pathway is restricted rather to the biosynthesis of pentoses and related metabolites which are shared with glycolysis.

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