

Case 35

Glucose-6-phosphate dehydrogenase activity and cell growth

Last modified 13 February 2004, K. Cornely, Providence College

Focus concept

The activity of the pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase has been found to be important in the regulation of cell growth.

Prerequisites

- C Pentose phosphate pathway (PPP) reactions
- C SDS-PAGE analysis
- C DNA replication
- C Signal transduction pathways involving tyrosine phosphorylation

Background

Numerous studies have shown that the intracellular redox potential of the cell is important to cell growth. Cellular redox potential can be determined by the amounts of the reduced coenzyme NADPH, a principal product of the oxidative branch of the pentose phosphate pathway (PPP). The investigators in the study presented here sought to demonstrate links between the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD) activity, cellular NADPH concentrations, and rates of cell growth. Previous studies have shown that the glucose-6-phosphate dehydrogenase enzyme can be activated on the order of minutes or even seconds, possibly through the action of growth factors that release a bound, inactive G6PD to the cytosol, where, via a mechanism that might involve tyrosine phosphorylation of a membrane-bound receptor, the unbound G6PD translocates to the cytosol and becomes active.

NADPH is important to the cell in a variety of ways. The reduced coenzyme can react with potential damaging oxidizing agents, ridding the cell of these agents before they can damage important cellular components. For example, hydrogen peroxide, H_2O_2 , may be reduced to water with concomitant oxidation of NADPH, as shown in Figure 35.1.

Notes on experimental technique:

1. Enzyme activity was measured by noting the increase in absorbance at 341 nm, as NADPH absorbs light at this wavelength and NADP^+ does not.
2. The Western blot is used to detect the presence (and relative amount) of a particular protein. Samples are first subjected to separation by SDS-PAGE, then the proteins in the gels are transferred to nitrocellulose paper. The paper is incubated with a specific antibody to the protein of interest; the antibody is tagged in some way to allow detection of the specific protein, which is visualized as a band. The darkness of the band is proportional to the amount of protein detected.
3. Incorporation of $[\text{}^3\text{H}]$ -thymidine into DNA is used as a measure of cell growth, since rapidly growing cells will replicate their DNA before undergoing cell division.

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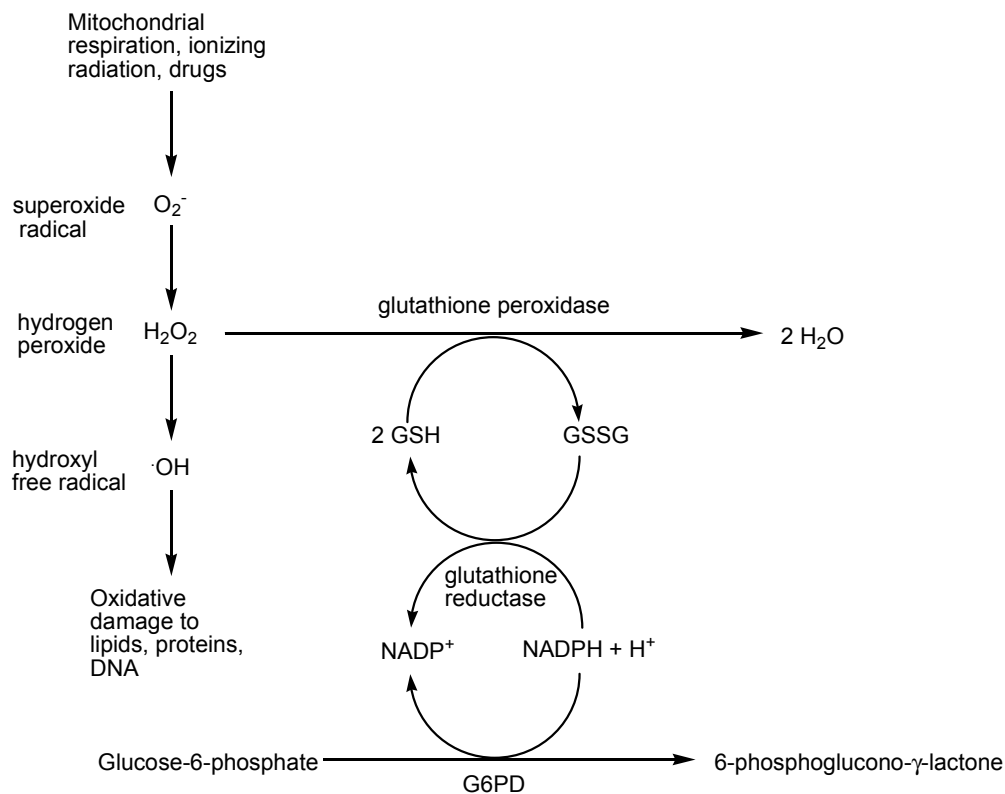


Figure 35.1: Detoxification of oxidizing species. Hydrogen peroxide is reduced to water by glutathione peroxidase with concomitant oxidation of reduced glutathione (GSH). The oxidized glutathione (GSSG) is converted back to the reduced form by glutathione reductase; NADPH is a required cofactor. Hydrogen peroxide can also be reduced to water via the catalase reaction (not shown); this reaction requires NADPH. (Adapted from Nelson, D. L., and Cox. M. M., (2000) *Lehninger Principles of Biochemistry*, Worth Publishers, NY, p. 560.

Questions

- Draw the reaction of the PPP catalyzed by glucose-6-phosphate dehydrogenase (G6PD). Why is this reaction important to the PPP?
 - Draw a diagram of the branch of the pentose phosphate pathway which involves G6PD. What are the two main products of this branch, and what is the importance of these products to the cell?
- List general cellular mechanisms that would affect the activity of G6PD. Which of these mechanisms would result in long-term (on the order of minutes or hours) vs. short term (minutes or seconds) regulation of the enzyme's activity?

3. In the first experiment reported in this study, the authors stimulated cell growth through a variety of means; then measured corresponding G6PD activity of cultured fibroblasts. (Cells grown in culture are typically grown in a medium containing 10% fetal calf serum, a medium rich in growth factors.) The investigators grew fibroblasts in the absence of serum (serum-starved) to serve as a control. Cells were then treated with 10% fetal calf serum followed by the serum-starvation treatment; other cells were not starved. The results are shown in Figure 35.2.

After the enzyme activity was measured, the cells were lysed and analyzed by Western blotting using a G6PD antibody. The results are of the Western blot are shown in Figure 35.3. What is your interpretation of these results? Be quantitative in your assessment of the data for both the activity assays and the Western blot. Why was the enzyme activity of PGD (6-phosphogluconate dehydrogenase) also investigated under the same conditions?

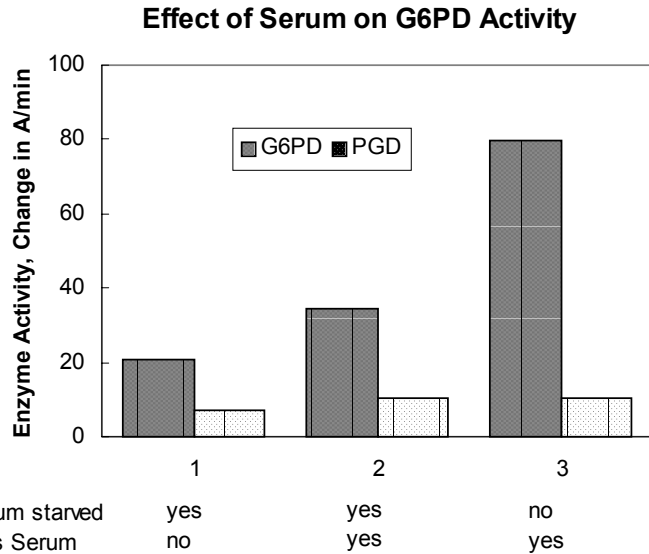


Figure 35.2: Fibroblasts were (1) serum-starved for 48 hours, (2) serum-starved for 48 hours and then stimulated with 10% calf serum, and (3) actively grown in medium containing 10% fetal calf serum. Enzyme activity was measured in lysates containing the same number of cells (from Tian, *et al.*, 1998).

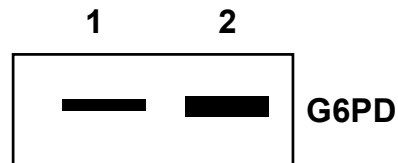


Figure 35.3: Western blot analysis of the same cell lysates as shown in Figure 35-1. Lane 1 shows serum-starved cells; Lane 2 is cells grown in the presence of serum (from Tian, *et al.*, 1998).

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4. It has been shown that dehydroepiandrosterone (DHEA) is an inhibitor of G6PD. The investigators incubated cells with DHEA, then lysed the cells and measured G6PD and PGD enzymatic activity in terms of the change in absorbance at 341 nm. In a separate experiment, the incorporation of [³H]-thymidine was measured in the presence of PGDF (platelet derived growth factor, which stimulates cell growth) and increasing concentrations of DHEA. The results are shown in Figures 35.4 and 35.5, respectively. A Western blot analysis was also carried out (data not shown), in which the investigators found that there was no change in enzyme protein in cells exposed to either PGDF, or DHEA, or both. What is your interpretation of the results shown in these two figures? Again, be quantitative in your assessment of the data.

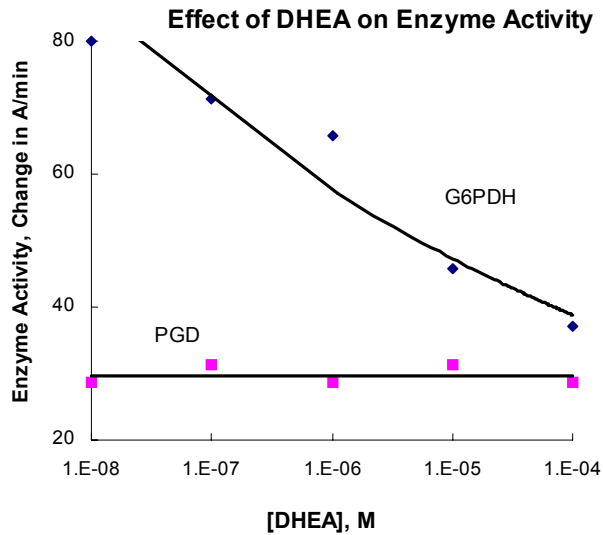


Figure 35.4: Dose-response curves of the effect of DHEA on G6PDH and PGD activity. Cell lysates were analyzed for enzyme activity in the presence of varying amounts of DHEA (from Tian, *et al.*, 1998).

5. Predict the effect of the NADPH/NADP⁺ ratio (as compared to controls) in cells under the following circumstances:
1. Withdrawal of serum.
 2. Addition of DHEA.
 3. Addition of H₂O₂.
 4. Withdrawal of serum and addition of H₂O₂.

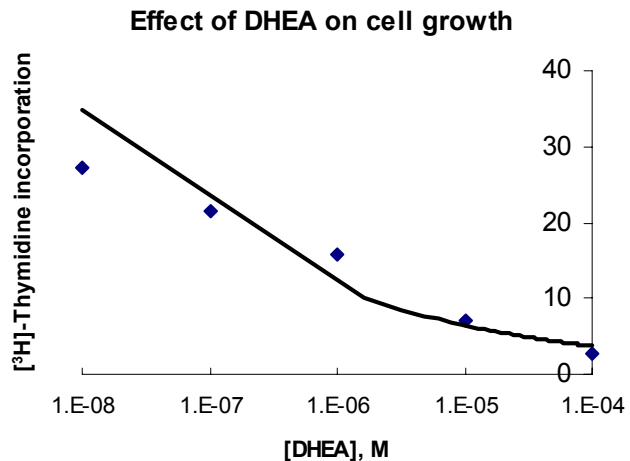


Figure 35.5: Serum-starved cells were treated with DHEA in the presence of PGDF for 12 hrs, then [³H]-thymidine was added and the cells were incubated for an additional 12 hrs (from Tian, *et al.*, 1998).

6. In order to more conclusively show a link between G6PD and cell growth, the investigators transfected a cell line, COS-7, with the G6PD gene. The transfected COS-7 cells showed a 2-3-fold increase in G6PD activity as compared to cells that hadn't been transfected with the gene. The authors of the study then investigated the effect of the inhibitor DHEA on these transfected cells by measuring the amount of [³H]-thymidine incorporation into COS-7 and control cells. The results are shown in Figure 35.6. What is your interpretation of these results? Again, be quantitative.

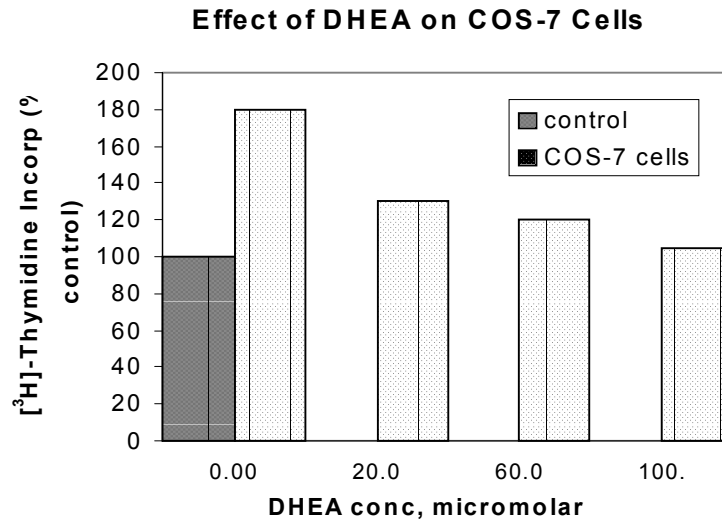


Figure 35.6: The effect of DHEA, as measured by [³H]-thymidine incorporation, was determined in COS-7 cells transfected with the G6PD gene, and compared to control cells (from Tian, *et al.*, 1998).

7. The authors of this study have shown that platelet derived growth factor (PDGF) stimulates the release of G6PD to the cytosol, and that this release depends on tyrosine phosphorylation of PDGF. They believe that it is likely that phosphatidylinositol-3-kinase (PI-3-kinase) and phospholipase C- β are involved. Draw a diagram that shows the sequence of events involved in the release and subsequent activation of G6PD.

Reference

Tian, W.-N., Braunstein, L. D., Pang, J., Stuhlmeier, K. M., Xi, Q.-C., Tian, X., and Stanton, R. (1998). *J. Biol. Chem.*, **273**, pp. 10609-10617.