THE PENTOSE PHOSPHATE PATHWAY: EVIDENCE FOR THE INDISPENSABLE ROLE OF GLUCOSE-PHOSPHATE ISOMERASE

Michael J. MORGAN
Department of Biochemistry, University of Leicester, Leicester, LE1 7RH, England

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1. Introduction

The pentose phosphate pathway plays an essential role in many cells and tissues providing a means for the generation of NADPH, pentose sugars and erythrose 4-phosphate.

It has been claimed that the classic reaction sequence of the pentose phosphate pathway as represented in most biochemistry textbooks requires major modification [1]. The classic scheme (F-scheme, [1]) is based on a number of observations including the labelling pattern of intermediates found following longer incubations of tissue extracts with specifically labelled [^{14}C]glucose [2]. The revised scheme (L-scheme [1]) is also based on the labelling pattern of such intermediates, but with shorter incubation periods. In the classic scheme glucose 6-phosphate (G6P) generated from glucose, is converted to ribulose 5-phosphate and thence is transformed, by the combined operation of an isomerase, epimerase, transaldolase and transketolase, to give fructose 6-phosphate (F6P) and glyceraldehyde 3-phosphate (which may be converted to F6P). The overall stoichiometry may be represented as:

$$6\ G6P + 12\ NADP^+ \rightarrow 5\ F6P + 6\ CO_2 +$$

$$12\ NADPH + 12\ H^+ + P_i$$

The cycle is completed by the isomerisation of F6P to G6P catalysed by glucose phosphate isomerase (GPI). In the L-scheme the final products are the same as in the classic scheme, although some of the intermediates (which include octulose mono- and bisphosphates) are different and there are additional enzymatic steps catalysed by aldolase a new epimerase and a new phosphotransferase; there is no role for transaldolase [1]. One crucial difference in the two pathways is the role of GPI: in the classic scheme the formation of G6P in the rearrangement reactions is entirely dependent on the activity of this enzyme. In the revised, L-scheme, G6P is formed directly (along with F6P) by the action of transketolase on erythrose 4-phosphate and octulose 8-phosphate. The formation of G6P in this scheme is thus not dependent on the activity of GPI. Chinese hamster cells devoid of GPI activity have been described [3,4]. Such mutants allow a direct test to determine which of the two pathways operate in these cells: cell extracts should convert ribose 5-phosphate (R5P) only to F6P if the classic scheme operates, but would convert R5P to G6P if the L-scheme operates. Here, I describe data consistent with the operation solely of the classic pathway.

2. Experimental

The origin and relevant characteristics of the cell lines used here are presented in table 1. Cells were grown in Ham's F12 medium supplemented with serum and antibiotics as in [5].

The conversion of ribose 5-phosphate to hexose phosphate by cell free extracts was measured in two ways:

(I) Cell extracts were prepared by freeze–thawing and a brief centrifugation step [3]. The reaction was monitored at 340 nm in a Gilford 240 spectrophotometer at room temperature. The reaction mixture (1 ml) contained: 100 μmol Tris–HCl (pH 7.5), 5.0 μmol $MgCl_2$, 50 μg thiamine pyrophosphate, 500 μg NADP, 10 μmol R5P,

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Table 1
Properties of cells used in this study

Cell line	Origin	Glucose phosphate isomerase act. (nmol . min^{-1} . mg protein^{-1})	Growth properties
CHO-K1	Chinese Hamster ovary [6]	194 [3]	glucose$^+$ ribose$^-$
R1.1.7	CHO-K1	10 [3]	glucose$^+$ ribose$^+$
023	Chinese Hamster lung [4]	1100 [4]	glucose$^+$ ribose$^-$
DS7	023	5 [4]	glucose$^+$ ribose$^-$

2.5 units of glucose 6-phosphate dehydrogenase (Sigma) and cell extract (40–150 μg protein). There was a substantial time lag before $NADP^+$ reduction was observed. When the reduction of $NADP^+$ ceased in the reaction mixtures containing wild-type extract (CHO-K1 and 023 cells), GPI (Sigma, 5 units) was introduced into the reaction mixtures containing extracts of R1.1.7 and DS7 cells (GPI mutants). An immediate and rapid reduction of $NADP^+$ then occurred. In all instances the reduction of $NADP^+$ was dependent on the presence of R5P, but not on the presence of thiamine pyrophosphate.

(II) Dialysed extracts of cells prepared as above were incubated for 3 h at 37°C in a reaction mixture (190 μl) containing: 25 μmol iminazole (pH 7.3), 1 μmol $MgCl_2$, 2 μmol R5P with and without the addition of thiamine pyrophosphate (100 μg) and extract (350 μg). The reaction was stopped by the addition of ice-cold 2 M perchloric acid. After 30 min on ice the precipitate was removed by centrifugation and the supernatant neutralized with K_2CO_3. After removal of the $KClO_4$ the supernatants were assayed for G6P and F6P using glucose 6-phosphate dehydrogenase and GPI. The reaction was dependent on the presence of R5P, but not thiamine pyrophosphate. Further details are as in [3].

3. Results and discussion

Table 1 illustrates some of the characteristics of the cells used in this study, other details are as in [3–5]. Both GPI mutants grow well on glucose and at similar rates to the wild-type cells [4,5] and both metabolise glucose essentially via the pentose phosphate pathway. Thus neither GPI mutant is able to oxidize [6-^{14}C]glucose to $^{14}CO_2$ but both readily oxodise [1-^{14}C]glucose to $^{14}CO_2$ and extracts of R1.1.7 and CHO-K1 cells have been shown to contain similar activities of the enzymes of the classic pentose phosphate pathway [5]. Both mutants have been shown to be essentially devoid of GPI activity in vitro [3,4]. This lesion may also be demonstrated to operate in vivo since mutant cells growing on glucose do not excrete lactic acid [3,4] and accumulate large quantities of G6P [4]. R1.1.7 cells growing on ribose do not accumulate G6P and can be shown to have an absolute requirement for inositol. Since G6P is the immediate precursor of inositol one may conclude that R1.1.7 cells are unable to synthesise G6P from ribose.

The conversion of R5P to G6P is shown in table 2. Extracts of CHO-K1 and 023 wild-type cells are able to convert R5P to G6P whereas R1.1.7 and DS7 extracts cannot but convert R5P only as far as F6P. If extracts of the GPI mutants are supplemented with commercial GPI then G6P is formed. Extracts of the GPI mutants, after incubation with R5P, contain

Table 2
Conversion of ribose 5-phosphate to hexose phosphates (nmol) in 3 h

Assay \ Cells	CHO-K1	R1.1.7	023	DS7
Method I	362	0	381	0
+ GPI	n.d.	339	n.d.	399
Method II	227	0	n.d.	n.d.
+ GPI	259	171	n.d.	n.d.

n.d., not done

approximately equivalent amounts of F6P as extracts of wild-type cells, similarly incubated, contain G6P. Thus the formation of G6P from R5P is totally dependent on the activity of GPI.

I conclude that in these two independently isolated GPI mutants, derived from entirely different Chinese hamster cell lines, the classic pentose phosphate pathway, and not the L-type pathway, operates. Similar findings have been made in extracts of GPI mutants of *Escherichia coli* (R. A. Cooper, personal communication). In [1]; it was claimed that the classic pathway operated in only fat pad of the 17 different animal and plant preparations examined. It is possible that the classic scheme will eventually need to be modified, however these data, which directly demonstrate that G6P formation is absolutely dependent on the activity of GPI urge caution and it would, therefore, seem premature to revise the usual textbook scheme of the pentose phosphate pathway.

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