THE REGULATION OF RIBOSE-5-PHOSPHATE ISOMERISATION IN *ESCHERICHIA COLI* K12

A.J. SKINNER and R.A. COOPER

Department of Biochemistry, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, England

Received 27 November 1970

1. Introduction

It seems likely that the non-oxidative reactions of the pentose phosphate pathway, acting in opposite directions, are responsible for both the biosynthesis and degradation of ribose-5-phosphate (R-5-P) in *E. coli* [1, 2]. However, such a dual role for a metabolic pathway is unusual and presents problems for the effective metabolic regulation of the two processes. In this paper we present evidence for a novel type of regulation which operates in this pathway, whereby a readily reversible reaction is catalysed unidirectionally. We show that two distinct forms of pentose phosphate isomerase (PPI) exist in *E. coli*, one responsible for the formation of R-5-P from ribulose-5-phosphate (Ru-5-P) and the other catalysing the conversion of R-5-P to Ru-5-P (see scheme 1). These findings are supported by a recent report that *E. coli* contains a form of PPI apparently specific for the conversion of R-5-P to Ru-5-P *in vivo* [3].

2. Materials and methods

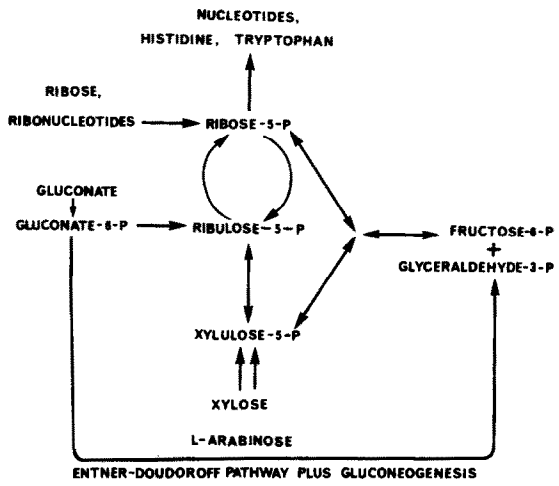
The organisms used were the *E. coli* K12 strains DF 2001 [4] and AB 2297 [5]. They were grown aerobically at 37° in "Oxoid" nutrient broth or appropriately supplemented minimal medium [6] containing the carbon source at 25 mM concentration. Where necessary ribose (2.5 mM final concentration) was included. Cells were harvested in the late logarithmic phase of growth, suspended in 50 mM tris HCl buffer pH 7.4 and disrupted by exposure to ultrasonic oscillations for 30 sec. The suspensions were centrifuged at 30,000 *g* for 15 min at 4° and the supernatants assayed for PPI. Ribokinase was assayed in the supernatants obtained after centrifugation of the suspensions at 160,000 *g* for 90 min [7]. Soluble protein was measured colorimetrically [8] using crystalline bovine serum albumin as the standard.

For the estimation of PPI the incubation mixtures at 37° contained, in 0.5 ml: sodium borate buffer pH 7.5 (25 μmoles), R-5-P (2.5 μmoles) and crude wild-type protein (5 μg) or crude mutant protein (80–500 μg). The Ru-5-P formed was measured colorimetrically [9] in a final volume of 1.8 ml. Under these conditions 1 μmole of Ru-5-P gave an absorbance of 5.0 units at 520 nm. Ribose permease was measured by the previously published method [10].

Ribose-requiring mutants were induced with ethyl methanesulphonate (EMS) [11] and selected with penicillin [12] for their inability to grow on a mixture of L-arabinose and glucose whilst retaining the ability to grow on glucose supplemented with ribose. Ribose-negative mutants were obtained after EMS mutagenesis by selection for their inability to grow on ribose whilst retaining the ability to grow on glucose.

3. Results

Mutants of DF 2001 were obtained that were unable to grow on gluconate, lactate, glucose, glycerol or succinate unless these compounds were supplemented with a small amount of ribose (or a compound such as adenosine or uridine which is



Scheme 1.

catabolised to R-5-P) but they could grow on ribose itself. These growth characteristics suggested that the mutants were unable to synthesise R-5-P from non-ribose precursors and the biochemical basis for this defect has been investigated in one such mutant, AS11.

The pleiotropic phenotype of AS11 was shown to be the result of a single mutation since revertants obtained from glucose, gluconate, lactate and succinate plates had regained, simultaneously, the ability to grow on the other carbon compounds in the absence of ribose.

Table 1
Effect of various growth conditions on the activity of PPI in DF 2001 and AS11.

Growth condition	nmoles R-5-P isomerised/min mg/protein in:	
	DF 2001	AS11
Ribose	2800	103
Nutrient broth	3040	17
Nutrient broth	520*	–
Nutrient broth + ribose	3240	107
Nutrient broth + ribose	–	43*
Nutrient broth + uridine	3200	111
Nutrient broth + adenosine	3970	144
Nutrient broth + xylose	3800	8
Nutrient broth + L-arabinose	3700	8
Nutrient broth + glucose	3700	3

PPI was assayed as described in the Methods but for those values marked * the R-5-P concentration was 0.5 mM.

Although AS11 contained 6-phosphogluconate dehydrogenase and could make Ru-5-P from 6-phosphogluconate (scheme 1) its inability to grow on gluconate in the absence of ribose suggested that it was unable to form R-5-P from Ru-5-P, that is it lacked PPI. When this enzyme was measured in cell-free extracts prepared from nutrient broth-grown cells it was found at very high activity in extracts prepared from DF 2001 but was barely detectable in extracts of AS 11 (table 1). That PPI was required for R-5-P formation was confirmed when considerable PPI activity was detected in the revertants of AS11 which no longer required ribose for growth.

Although the virtual absence of PPI would explain the inability of AS11 to make R-5-P from non-ribose precursors it was apparently at variance with the ability of AS11 to grow on ribose since PPI is believed to be necessary for ribose catabolism [1]. Accordingly, cell-free extracts prepared from ribose-grown AS11 were assayed for PPI activity. Table 1 shows that in this case AS11 had significant PPI activity, suggesting that there was a second PPI, the formation of which was elicited by ribose. This view was confirmed by the assay of PPI activity in cell-free extracts prepared from AS11 grown on variously supplemented nutrient broth. As can be seen from table 1, the presence of glucose, xylose or L-arabinose did not cause any increase in the PPI activity of AS11 but the addition of ribose, adenosine or uridine resulted in the formation of the enzyme to the same extent as in ribose-grown cells.

Table 2
Effect of various growth conditions on the amounts of ribose permease, ribokinase and PPI in *E. coli* strain AS11.

Growth condition	Activity of:		
	Ribose[a] permease	Ribo-kinase[b]	PPI[c]
Nutrient broth	2.0	31.6	16.7
Nutrient broth + ribose	8.3	97.7	107
Nutrient broth + adenosine	2.0	31.1	144
Nutrient broth + uridine	2.8	34.4	111

[a]nmoles ribose incorporated/min/mg dry wt at 25°.
[b]nmoles ribose phosphorylated/min/mg protein at 30°.
[c]nmoles R-5-P isomerised/min/mg protein at 37°.

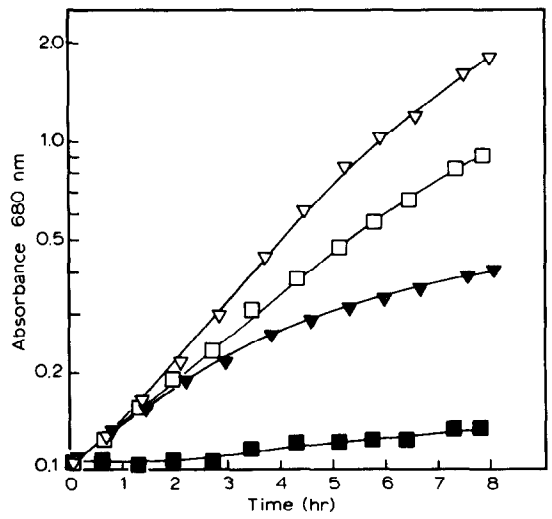


Fig. 1. Growth of AS11 on glucose in the presence (open symbols) and absence (filled symbols) of ribose. The cells were pregrown on lactate/ribose (▽) and glucose/ribose (□).

This pattern of induction suggested that the second form of PPI was involved in the catabolism of R-5-P. Although its specific activity (105 nmoles R-5-P isomerised/min mg/protein) was low compared to the form of PPI involved in the gluconeogenic formation of R-5-P, the 'catabolic' PPI was comparable in activity to two other enzymes involved in ribose degradation, ribose permease and ribokinase (table 2). It is interesting to note that the induction of the 'catabolic' PPI is apparently distinct from the induction of ribose permease and ribokinase since adenosine and uridine which induce the formation of the 'catabolic' PPI have no effect on the amounts of ribose permease and ribokinase (table 2).

The existence of a form of PPI necessary for R-5-P catabolism has also been suggested by David & Wiesmeyer [3]. This view has been strengthened by a study of the properties of a ribose-negative mutant (RC2) isolated from strain AB 2297. RC2 was unable to grow on uridine or adenosine which distinguished it from ribokinase and ribose permease-negative mutants [10], but like them it grew normally on other pentoses. Since it grew normally on D-arabinose which is catabolised via D-ribulose-5-P [13] it seemed that RC2 was unable to form Ru-5-P from R-5-P, that is, it lacked the 'catabolic' PPI. When PPI activity was measured in extracts prepared from nutrient broth *plus* ribose-grown RC2 and AB 2297, equal and high activities were present in both extracts. However, since the ribose-induced PPI in AS11 was less than 5% of the total PPI of DF 2001 the absence of such a small fraction of the total activity is likely to go undetected. That the constitutive "biosynthetic" PPI accounts for greater than 95% of the total PPI of AB 2297 was confirmed by the properties of a ribose-requiring mutant (AS131) isolated from AB 2297. Cell-free extracts prepared from AS131 grown on nutrient broth were almost totally devoid of PPI activity but similar extracts prepared after growth in the presence of ribose contained the second PPI activity. Thus the various properties of ribose-negative mutant RC2 are consistent with the absence of the catabolic PPI [3] but a means of separating the two isomerases must be found before this can be demonstrated directly.

In contrast to this observation that only one form of PPI can convert R-5-P to Ru-5-P, *in vivo*, see also [3], we have shown that the 'catabolic' isomerase can convert Ru-5-P to R-5-P *in vivo*. Thus, when AS11 is fully induced for the 'catabolic' PPI by prior growth on lactate supplemented with ribose it can grow well on glucose in the absence of ribose for just over one generation (fig. 1). However, when it is pregrown on glucose supplemented with ribose it contains very little PPI (due to glucose repression of the ribose catabolic enzymes) and fails to grow on glucose unless ribose is included (fig. 1). Despite this finding the inducible nature of the 'catabolic' PPI means that it is not involved in R-5-P biosynthesis *in vivo*.

4. Discussion

From the results presented here it is clear that two pentose phosphate isomerases with distinct physiological roles are present in the two *E. coli* K12 strains studied. The inducible nature of one of the isomerases implies that in the absence of ribose the cells contain only one PPI, which functions in R-5-P biosynthesis. During growth on ribose (or compounds that can give rise to R-5-P) the inducible PPI would be formed and it seems likely that only this inducible

PPI can convert R-5-P to Ru-5-P *in vivo*, see also [3]. The basis of this specificity is as yet unknown. However, our studies show that the 'biosynthetic' PPI is present in great excess: at a R-5-P concentration of 0.5 mM (K_m for 'catabolic' PPI is 0.95 mM R-5-P; K_m for 'biosynthetic' PPI is 6.2 mM R-5-P) the 'biosynthetic' PPI activity is still 12 times that of the 'catabolic' PPI (table 1). It is thus unlikely that the intracellular concentration of R-5-P would permit only the 'catabolic' enzyme to function, as has been suggested by David & Wiesmeyer [3]. Work in progress may provide an answer to this problem.

Acknowledgements

Our thanks are due to Professor H.L. Kornberg, F.R.S., for helpful discussions and to the Science Research Council who supported this work under grant number B/SR/7309.

References

[1] H.Z. Sable, Advan. Enzymol. 28 (1966) 391.
[2] R. Caprioli and D. Rittenberg, Proc. Natl. Acad. Sci. U.S. 61 (1968) 1422.
[3] J. David and H. Wiesmeyer, Biochim. Biophys. Acta 208 (1970) 56.
[4] D.G. Fraenkel, J. Bacteriol. 95 (1968) 1267.
[5] H.L. Kornberg and Janet Smith, Nature 224 (1969) 1261.
[6] J.M. Ashworth and H.L. Kornberg, Proc. Roy. Soc. London Ser. B. 165 (1966) 179.
[7] Anne Anderson and R.A. Cooper, Biochim. Biophys. Acta 177 (1969) 163.
[8] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J Randall, J. Biol. Chem. 193 (1951) 265.
[9] B. Axelrod, in: Methods in Enzymology, Vol. I, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York, 1955) p. 363.
[10] Anne Anderson and R.A. Cooper, J. Gen. Microbiol. (1970) in press.
[11] E.C.C. Lin, S.A. Lerner and S.E. Jorgensen, Biochim. Biophys. Acta 60 (1962) 442.
[12] L. Gorini and H. Kaufman, Science 131 (1960) 604.
[13] S.S. Cohen, J. Biol. Chem. 201 (1953) 71.