Journal of Industrial Microbiology (1996) 17, 458–462

Basic and applied aspects of metabolic diversity: the phosphoenolpyruvate node

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The phosphoenolpyruvate (PEP) node represents a metabolic crossroad where carbon is distributed into several metabolic pathways. This node is specially important for the industrial production of several metabolites. Depending on the organism and its habitat, the enzymes that utilize PEP are regulated by different effectors, and each branch of the node is important in PEP consumption. In this review we will focus our attention on the metabolic diversity of this node.

Keywords: central metabolism; glycolysis; bacterial metabolism; metabolic engineering

Introduction

Life processes depend on the different functions that proteins, nucleic acids and carbohydrates are capable of providing. Despite their complexity, these macromolecules are constructed by joining together a variety of simpler units. These units, or building blocks, are obtained through diverse cellular catabolic and biosynthetic routes. However, regardless which metabolic routes an organism uses, all cells synthesize the following twelve precursor metabolites: glucose-6-phosphate, fructose-6-phosphate, ribose-5-phosphate, erythrose-4-phosphate, triose-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, pyruvate, acetyl CoA, α-ketoglutarate, succinyl CoA and oxaloacetate.

During balanced growth, each cell-type has evolved control mechanisms to guarantee that its major components remain relatively proportional to one another. These mechanisms should operate to assure the proper distribution of precursors to all biosynthetic routes. Furthermore, these control mechanisms need to be flexible enough to assure that under non-balanced growth conditions, an adequate distribution of metabolites is maintained. One way to fulfill both requirements has been through the activation and/or inhibition of key enzymes by allosteric effectors. These effectors can be products of energy metabolism such as AMP, ATP or NADH. Other intermediary metabolites like pyruvate (PYR), phosphoenolpyruvate (PEP), acetyl-CoA and aspartate control specific points of some of the metabolic routes involved.

For these reasons, it is important to understand how different microorganisms regulate fluxes through their primary metabolic routes. It is well known that, depending on the microorganism and its habitat, metabolic flux patterns can be different. For the applied sciences, knowledge about cellular metabolic fluxes and regulation of end-product formation is especially important at the present time. The diversity of microorganisms offers an enormous source of enzymes with different properties that may be used to improve commercial products or production processes. Furthermore, enzymes may be modified or new genes can be recruited to construct new metabolic pathways.

In this paper we will review some aspects of the enzymes that utilize PEP as substrate. PEP is a key intermediate for the biosynthesis of several important compounds. This strategic node has been studied extensively in *Corynebacterium glutamicum* [12,14,32] and in *Escherichia coli* [27,28]. From the enormous amount of information that exists about central metabolism, we have selected those references that make our points clear. This is not an exhaustive review, but one which hopefully encourages more research into bacterial metabolism, and more interest in taking advantage of the immense supply of enzymes and metabolic strategies that Nature can provide.

Two questions will be addressed: how diverse are the enzymes that participate in the PEP node?; and how can we benefit from this diversity? We will focus our discussion basically on bacteria, but, in a few instances, we will also use such examples as plant systems. Among the bacteria we will initially focus our discussion on *E. coli* metabolism, considering that no other microbe has been studied in such detail, where more than 80% of the cell's metabolic routes are known, and more than 60% of its genome sequenced. From there, discussion will be expanded to some other systems.

The phosphoenolpyruvate node

Glucose can be used by the cell to provide all the carbon skeletons needed to synthesize the twelve precursor metabolites mentioned, which are formed through the concerted action of the glycolytic, pentose phosphate and tricarboxylic acid (TCA) pathways [20]. Several biomolecules are derived from PEP (Figure 1), and it is one of the most important nodes for carbon distribution in all living cells. In *E. coli*, PEP is utilized basically by the reactions summarized in Figure 2. The phosphotransferase transport system (PTS) is the major PEP consumer and PYR producer, while

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Received 5 March 1996; accepted 20 June 1996

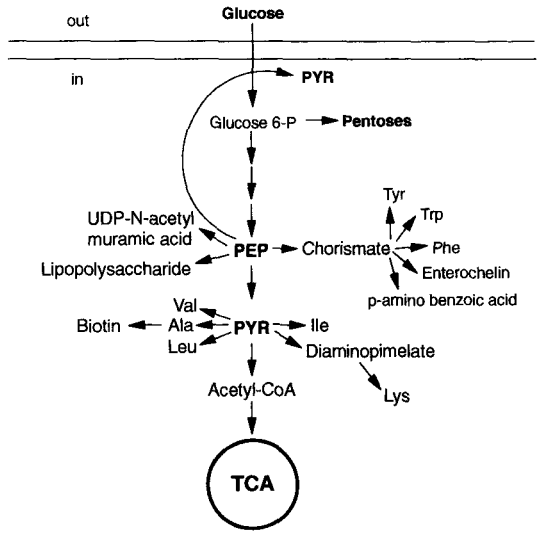

Figure 1 Major metabolic pathways in *Escherichia coli* that, directly or indirectly, utilize phosphoenolpyruvate.

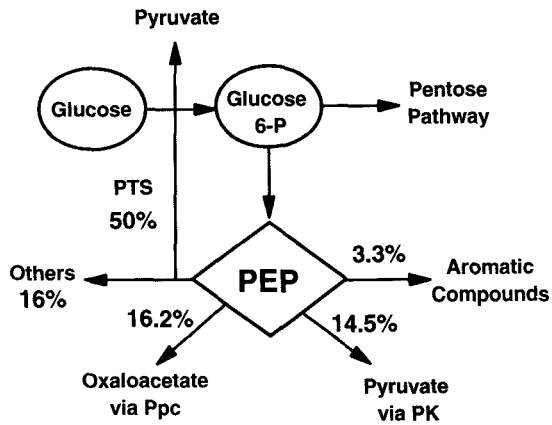

Figure 2 Distribution of phosphoenolpyruvate (PEP) in *Escherichia coli*. The percentages were calculated from data reported by Holms [11].

the phosphoenolpyruvate carboxylase (Ppc) and pyruvate kinase (PK) enzymes consume 16.2% and 14.5% of PEP, respectively. Only 3.3% of the PEP is diverted to aromatic compound synthesis [11].

The fact that an important portion of the studies on metabolism has been done in yeast, or in *E. coli*, could lead to the conclusion that central metabolism is based on the glycolytic or Embden–Meyerhof–Parnas (EMP) pathway. However, there are two alternative routes for glucose utilization: the hexose monophosphate shunt (HMS) and the Entner–Doudoroff (ED) pathways [20]. The HMS pathway oxidizes glucose to CO_2 and glyceraldehyde-3-phosphate, which in turn, can be directed into the TCA cycle via pyruvate by the EMP enzymes, providing NADPH and precursors for nucleotide and aromatic compounds biosynthesis. In bacteria like *E. coli*, this metabolic route consumes around 30% of the glucose-6-phosphate [11]. In certain microorganisms like *Thiobacillus novellus* and *Brucella abortus*, which lack the key enzymes of the EMP or ED pathways, the HMS route has a central role in glucose assimilation [20]. Likewise, it was recently demonstrated that in *Corynebacterium glutamicum*, the HMS pathway also has a major role, even though this microorganism has a functional EMP pathway [18].

Another route for glucose assimilation is the ED pathway which is widely distributed among bacteria. As in the HMS, the first intermediate in the ED pathway is 6-phosphogluconate. Two additional enzymes metabolize 6-phosphogluconate into pyruvate and glyceraldehyde-3-phosphate. In many *Pseudomonas* species and in *Zymomonas*, the Entner–Doudoroff pathway is the main glucose assimilatory route [15,35]. In microorganisms that utilize the HMS or ED metabolic routes to assimilate glucose, the importance of the PEP node and the allosteric regulation of the enzymes involved, will be different than in cells using the EMP pathway.

The phosphotransferase transport system

When *E. coli* is grown in minimal media with glucose as the only carbon source, the carbohydrate is transported inside the cell by the PTS. Concomitantly to its transport, glucose is phosphorylated to glucose-6-phosphate, the first intermediate of the glycolytic pathway (Figure 1). The PTS is also involved in transport and phosphorylation of a large number of carbohydrates, in signal transduction during chemotaxis toward certain carbohydrates, and in the global regulation of many metabolic pathways [30].

PTS is an extremely efficient uptake system; for example, if a transportable sugar is present in the medium at a concentration as low as 0.1 μM, PTS has the theoretical potential to accumulate the sugar against the concentration gradient up to 100 mM [26]. Such transport systems are essential for microorganisms living in media where carbohydrates are scarce. On the other hand, microorganisms that live in sugar-rich environments do not necessarily have PTS. For example, *Zymomonas mobilis* has a constitutive low-affinity high velocity facilitated diffusion system [5]; glucose is phosphorylated by glucokinase and metabolized through the ED pathway. Studies of the different properties of enzymes involved in glucose metabolism in *Zymomonas* have shown that they are operating at their near-maximal capacity, with no substantial allosteric control of the key enzymes [36].

The fact that PTS consumes at least 50% of the PEP made from glucose represents an important problem from the industrial point of view, since it decreases the availability of this intermediate for other reactions, such as aromatic compound synthesis. For example, it has been calculated that for tryptophan (Trp) synthesis, the achievable yield from glucose in a strain with an operating PTS (ie 1 mole of PEP per mole of glucose assimilated) is 20%. On the contrary, if PEP-consuming PTS was not involved and both PEP moles were available for Trp synthesis, the yield could be increased up to 41.8% [9]. This example clearly shows the importance of increasing the intracellular PEP availability for the synthesis of commercially important molecules. For example, it was demonstrated recently that an *E. coli* mutant devoid of the PTS, but capable of glucose transport by a non-PEP consuming mechanism, was able to redirect 50% more of its PEP into the aromatic aminoacid pathway [7]. These results demonstrate that it is possible

to exploit the metabolic diversity of the cell in order to construct mutants with new metabolic capabilities.

An alternative approach to the same problem was performed by exploiting the metabolic diversity among two different bacteria. In this system, the successful transfer and expression of the glucose permease and glucokinase structural genes from *Z. mobilis* in *E. coli pts* mutants, provided a new functional pathway for glucose uptake and phosphorylation. In the same report, it was suggested that the permease and glucokinase genes from *Z. mobilis*, could provide an alternative or supplemental route for glucose entry into glycolysis in other microorganisms [31].

Phosphoenolpyruvate carboxylase

Phosphoenolpyruvate carboxylase (Ppc) catalyzes CO_2-fixation on PEP yielding oxaloacetate. In most bacteria, green algae and in many plants, the major physiological role of this enzyme is to replenish the TCA cycle [3,14,25]. According to their properties, the Ppc enzymes have been classified in three types by Utter and Kolenbrander [34]; class 1 comprises those enzymes which are subject to both activation and inhibition, class 2 includes those proteins subject only to inhibition, and class 3 those enzymes that are subject neither to activation nor inhibition. For class 3, however, there are preliminary studies suggesting that in some cases reduced NADH is an activator of the enzyme [23].

The Ppc from *E. coli*, *S. thyphimurium*, *P. fluorescens* and several other organisms included in class 1, are activated by acetyl-CoA and inhibited by aspartate. Activation of the class 1 enzyme by ADP has also been reported in *P. citronellonis* and in *A. vinelandii*. However, ADP does not seem to affect Ppc from *E. coli* [24]. Nevertheless, the *E. coli* enzyme is allosterically regulated by multiple effectors such as fructose 1,6-diphosphate, GTP, certain long-chain-fatty acids and malate [4]. There are also reports describing that, among several other guanine nucleotides such as, GTP, GDP, GMP and ppGpp, the former is the most potent activator of the *E. coli* Ppc [33].

In contrast, the Ppc from *Z. mobilis* seems to be different and is not affected by acetyl Co-A. The enzyme is, however, inhibited competitively by intermediates of the TCA cycle, especially citrate, α-ketoglutarate and aspartate [4].

As in many mesophilic bacteria, the Ppc enzyme from *T. aquaticus* requires Mg^{2+}, and in the absence of acetyl Co-A, the enzyme is inactive. However, fructose-1,6-diphosphate, which activates several of the Ppc enzymes in mesophilic microbes, has no effect on Ppc action in *T. aquaticus* [3].

A NADH-activated form of Ppc has been reported in a *Pseudomonas* strain grown on methylamine. This enzyme is not activated by acetyl Co-A, ADP, GDP, or a wide variety of other metabolites [23].

It is generally assumed that in several bacterial species, the Ppc has an anaplerotic role, replenishing the tricarboxylic acid cycle (TCA) by supplying oxaloacetate directly from PEP. However, in the Chlorobiaceae, a group of photosynthetic bacteria, known also as the green sulfur bacteria, Ppc has a key role, participating actively in CO_2 fixation. These bacteria use a unique reductive TCA cycle that converts CO_2 into acetyl CoA, as shown in Figure 3. This pathway is unique in the sense that it is a tricarboxylic acid cycle that runs in the reverse direction. However, even though several of the TCA cycle reactions are reversible, three enzymes must be changed in order to drive the cycle in the reverse, or reductive direction [10]. Furthermore, the reductive TCA cycle has been proposed to be the ancestor of all carbon fixation pathways [36], and the evolutionary precursor of the oxidative TCA cycle [17,37].

The analysis of Ppc from plants also offers a rich source of diversity. One of the major characteristics of plants is their ability to fix atmospheric CO_2; the CO_2 fixation can be accomplished in two ways:

(1) In the Calvin Cycle or reductive pentose phosphate cycle, ribulose-1-5, biphosphate reacts with CO_2 to produce two molecules of glyceraldehyde-3-phosphate (PGA) in a reaction catalyzed by ribulose biphosphate carboxylase (RuDPCase). Because the initial carbon dioxide fixation product is a three-carbon compound (PGA), plants with this fixation pattern are often referred to as C_3 plants. The Calvin cycle occurs in many monocotyledons and dicotyledons, including such plants as wheat, rice, sugar beet, spinach, soybeans and tobacco [14].
(2) Another pattern of carbon dioxide assimilation features the production of a four-carbon compound as the CO_2-fixation product. Plants with this pathway are known as C_4 plants. In this type of plants, CO_2 reacts with PEP in the presence of Ppc to form oxaloacetate, where Ppc plays the key role of CO_2 assimilation, as does RuBPC in C_3 plants.

It is evident that the allosteric regulation of the Ppc from Chlorobiaceae and C_4 plants, should be different from the ones in microbes where its role is merely anaplerotic. Based on these data, and from the applied research point of view, it is possible to speculate that the use of a Ppc enzyme from C_4 plants or Chlorobiaceae sources, could be an interesting approach to direct more carbon into oxaloacetate for the overproduction of aspartate, lysine, threonine, methionine and isoleucine.

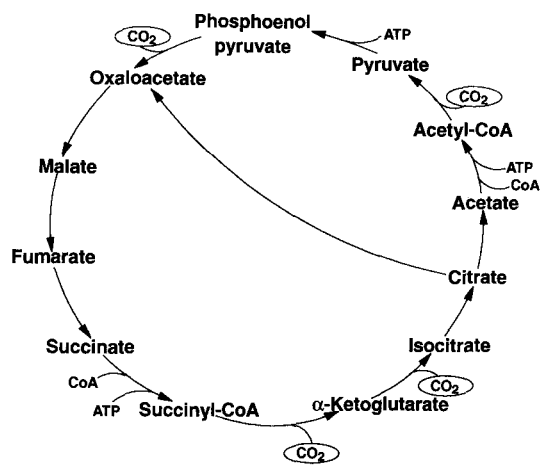

Figure 3 The reductive tricarboxylic acid cycle of the Chlorobiaceae. Adapted from [10 and 20].

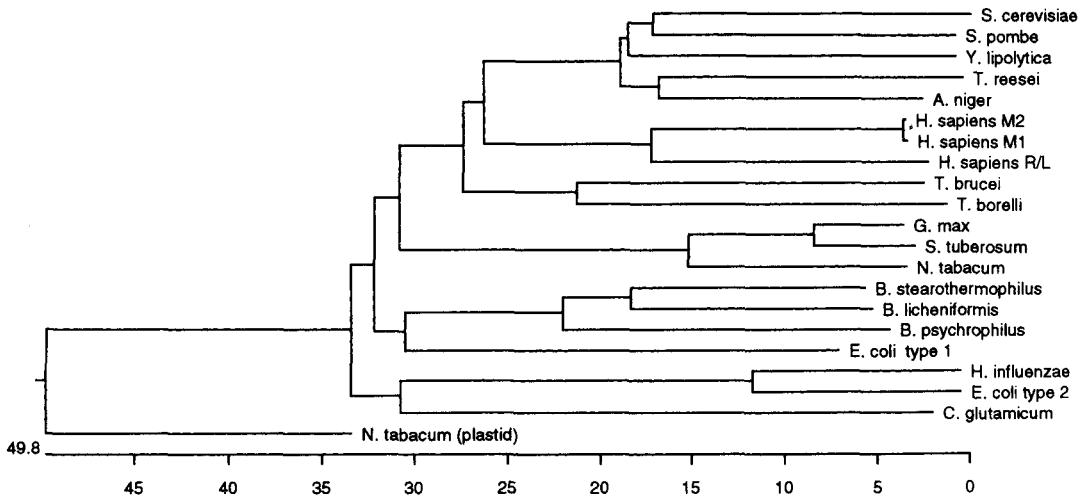

Figure 4 Phylogenetic tree of twenty one pyruvate kinases from different sources. The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences. The tree was constructed using the Lasergene software (DNASTAR Inc, Madison, WI, USA).

Pyruvate kinases

Pyruvate kinases (PKs) catalyze the conversion of PEP into pyruvate coupled to the synthesis of one molecule of ATP, as shown in Figure 1. This reaction is the last step of the glycolytic pathway and it is irreversible under physiological conditions. Together with phosphofructokinase (PFK), PK plays a major role in glycolysis regulation [8]. No other enzyme of central metabolism has received so much attention as the PK. More than 30 PK structural genes, from different sources, have been cloned and sequenced. In many cases, the enzymes have been purified and some biochemical and kinetic parameters have been determined. One of the reasons for the interest in PK is that a deficiency in this enzyme may be the most common cause of hereditary nonspherocytic hemolytic anemia in humans [1]. Besides this anthropocentric reason, the PK enzymes provide an attractive model to understand some aspects of allosteric regulation, as well as the modulation of glycolysis.

Most bacterial species have one PK. However, *E. coli* and *S. typhimurium* have two PKs, classified as types 1 and 2 based on their allosteric regulation. In PK type 1, allosteric regulation is achieved by binding fructose-1,6-biphosphate (FBP), resulting in increased enzyme activity. Without FBP or in the presence of ATP the enzymatic activity is lower. The allosteric regulators of type 2 PK are AMP and ribose-5-phosphate. The relative roles of these two enzymes have been studied in *E. coli* [29].

The crystal structure of the PK type 1 from *E. coli* has been determined in the absence of PEP, providing the tridimensional structure of an enzyme with a low level of activity. Another well known PK is the mammalian muscle M1 enzyme [13,21]. Interestingly, this enzyme has no allosteric regulation and its crystallization provides the structure of a PK on its active conformation. The comparison of both structures shows that in most parts, they are very similar. However, for the transition from the inactive to the active form, a complicated set of domain and subunit motions need to be induced by the allosteric regulators [19].

Along these lines, there have been some efforts to change the allosteric regulation of the PK from *B. stearothermophilus*. For this purpose, amino acid sequence comparisons between allosteric and non-allosteric PKs from various sources, in conjunction with homology modelling of *B. stearothermophilus* PK on mammalian muscle crystal structures, have been used as criteria to predict regions implicated in allosteric regulation [16,22].

These two examples clearly show the advantage of comparing the structural and biochemical diversity among enzymes from different sources, to understand allosteric regulation or predict the type of changes that need to be introduced to create enzymes more suitable for certain applications.

Finally, we point out that progress on DNA sequencing methodologies, provides an enormous amount of new sequences constantly. In this sense, the abundance of amino acid sequences reported can complicate the identification of potentially interesting enzymes. For example, it is common to see reports of the sequence of a new PK enzyme. Normally, this sequence is compared with the data banks and a similarity percentage is calculated. This could lead to the notion that this new PK is just 'another one'. However, with the same data, it is possible to construct a phylogenetic tree, which in turn, could provide another type of information [6]. In Figure 4 we present a phylogenetic tree constructed from the alignment of 21 known PK sequences. As can be seen, the PK from *C. glutamicum* is more related to the type 2 PKs than to the type 1 enzymes of *E. coli*. Interestingly, it was previously assumed that the former PK was more closely related to the type 1 PKs [12]. This information could be useful if some property of a particular PK needs to be changed. In this case, in order to facilitate the identification of targets for mutagenesis, it could be more advantageous to compare the amino acid sequences of the closest relatives.

It is also evident from Figure 4 that the PK from the *N. tabacum* plastid is more closely related to the prokaryotic enzymes than to the one located in its own cytosol. In this sense, it would be interesting to study the biochemical

properties of the PKs from plastids, because they have been under different selection pressures and especially because, in this organelle, PK participates in a glycolytic pathway that supplies substrates and cofactors for fatty acid biosynthesis [2].

Conclusion

We have reviewed several aspects of the different enzymes that utilize PEP as a substrate. Depending on the organism, these enzymes are regulated by different effectors in several metabolic pathways. The PEP node represents a strategic metabolic crossroad where carbon skeletons are distributed and directed into various metabolic pathways for the biosynthesis of many important compounds. Each cell has evolved sophisticated mechanisms to assure the proper distribution of the precursors to all biosynthetic routes and for the applied sciences the possibility of modifying these control mechanisms, could yield important rewards.

In order to reduce the cost of industrial biosynthetic production of several important compounds, such as the aromatic amino acids, it is desirable to increase the flux of certain carbon skeletons into and through the aromatic compound pathway. This has been accomplished by using cells carrying specific mutations and amplified genes that direct more PEP into this pathway [7]. These results clearly indicate that it is possible to exploit metabolic diversity to design and construct organisms with novel properties.

References

1 Barociani L and E Beutler. 1993. Analysis of pyruvate kinase-deficiency mutations that produce nonspherocytic hemolytic anemia. Proc Natl Acad Sci USA 90: 4324–4327.
2 Blakeley S, S Gottlob-McHugh, J Wan, L Crews, B Miki, K Ko and DT Dennis. 1995. Molecular characterization of plastid pyruvate kinase from castor and tobacco. Plant Mol Biol 27: 79–89.
3 Bridger GP and TK Sundaram. 1976. Occurrence of phosphoenolpyruvate carboxylase in the extremely thermophilic bacterium *Thermus aquaticus*. J Bacteriol 125: 1211–1213.
4 Bringer-Meyer S and H Sahm. 1989. Junctions of catabolic and anabolic pathways in *Zymomonas mobilis*: phosphoenolpyruvate carboxylase and malic enzyme. Appl Microbiol Biotechnol 31: 529–536.
5 DiMarco AA and AH Romano. 1985. D-Glucose transport system of *Zymomonas mobilis*. Appl Environ Microbiol 49: 151–157.
6 Doolittle RF. 1986. Of URFS and ORFS. A Primer on How to Analyze Derived Aminoacid Sequences. pp 40–47, University Science Books, California.
7 Flores N, J Xiao, A Berry, F Bolivar and F Valle. 1996. Pathway engineering for the production of aromatic compounds in *Escherichia coli*. Nature Biotechnol 14: 620–623.
8 Fraenkel DG. 1992. Genetics and intermediary metabolism. Annu Rev Genet 26: 159–177.
9 Frost J and J Lievense. 1994. Prospects for biocatalytic synthesis of aromatics in the 21st century. New J Chem 18: 341–348.
10 Gottschalk G. 1986. Bacterial Metabolism. Springer-Verlag, New York.
11 Holms W. 1986. The central metabolic pathways of *Escherichia coli*: relationships between flux and control at a branch point; efficiency of conversion to biomass, and excretion of acetate. In: Current Topics in Cellular Regulation (Horecker BL and ER Stadtman, eds) , pp 69–105, Academic Press, NY.
12 Jetten MSM, GA Pitoc, MT Follettie and AJ Sinskey. 1994. Regulation of phospho(enol)-pyruvate- and oxaloacetate-converting enzymes in *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 41: 47–52.
13 Larsen TM, LT Laughlin, HM Holden, I Rayment and GH Reed. 1994. Structure of rabbit muscle pyruvate kinase complexed with Mn^{2+}, K^+, and pyruvate. Biochemistry 33: 6301–6309.
14 Lepiniec L, J Vidal, R Chollet, P Gadal and C Crétin. 1994. Phosphoenolpyruvate carboxylase: structure, regulation and evolution. Plant Science 99: 111–124.
15 Lessie TG. 1984. Alternative pathways of carbohydrate utilization in pseudomonads. Ann Rev Microbiol 38: 359–387.
16 Lovell SC, AH Mullick, D Walker, A Rawas and H Muirhead. 1995. Structural and mutagenesis studies on the cooperativity of pyruvate kinase. Protein Engineering 8: 62.
17 Maden EBH. 1995. No soup for starters?; autotrophy and the origins of metabolism. Trends Biochem Sci 20: 337–341.
18 Marx A, AA de Graaf, W Wiechert, L Eggeling and H Sahm. 1996. Determination of fluxes in the central metabolism of *Corynebacterium glutamicum* by nuclear magnetic resonance spectroscopy combined with metabolite balancing. Biotechnol Bioeng 49: 111–129.
19 Mattevi A, G Valentini, M Rizzi, ML Speranza, M Bolognesi and A Coda. 1995. Crystal structure of *Escherichia coli* pyruvate kinase type I: molecular basis of the allosteric transition. Structure 3: 729–741.
20 Moat AG and JW Foster. 1988. Microbial Physiology. John Wiley & Sons, New York.
21 Muirhead H, DA Clayden, D Barford, CG Lorimer, LA Fothergill-Gilmore, E Schiltz and W Schmitt. 1986. The structure of cat muscle pyruvate kinase. EMBO J 5: 475–481.
22 Mullick AH, SC Lovell, D Walker and H Muirhead. 1995. Engineering the allosteric regulation of pyruvate kinase. Protein Engineering 8: 62.
23 Newaz SS and LB Hersh. 1975. Reduced nicotinamide adenine dinucleotide-activated phosphoenolpyruvate carboxylase in *Pseudomonas* MA: potential regulation between carbon assimilation and energy production. J Bacteriol 124: 825–833.
24 O'Brien RW, DT Chuang, BL Taylor and MF Utter. 1977. Novel enzymic machinery for the metabolism of oxaloacetate, phosphoenolpyruvate, and pyruvate in *Pseudomonas citronellolis*. J Biol Chem 252: 1257–1263.
25 O'Leary MH. 1982. Phosphoenolpyruvate carboxylase: an enzymologist's view. Ann Rev Plant Physiol 33: 297–315.
26 Parr TR and MH Saier. 1992. The bacterial phosphotransferase system as a potential vehicle for entry of novel antibiotics. Res Microbiol 143: 443–447.
27 Patnaik R and JC Liao. 1994. Engineering of *Escherichia coli* central metabolism for aromatic metabolite production with near theoretical yield. Appl Environ Microbiol 60: 3903–3908.
28 Patnaik R, R Spitzer and JC Liao. 1995. Pathway engineering for production of aromatics in *Escherichia coli*: confirmation of stoichiometric analysis by independent modulation of AroG, TktA, and Pps activities. Biotechnol Bioeng 46: 361–370.
29 Ponce E, N Flores, A Martínez, F Valle and F Bolívar. 1995. Cloning of the two pyruvate kinase isoenzyme structural genes from *Escherichia coli*: the relative roles of these enzymes in pyruvate biosynthesis. J Bacteriol 177: 5719–5722.
30 Postma PW, JW Lengeler and JR Jacobson. 1993. Phosphoenolpyruvate carbohydrate phosphotransferase systems of bacteria. Microbiol Rev 57: 543–594.
31 Snoep J, N Arfman, L Yomano, R Fliege, T Conway and L Ingram. 1994. Reconstitution of glucose uptake and phosphorylation in a Glucose-negative mutant of *Escherichia coli* by using *Zymomonas mobilis* genes encoding the glucose facilitator protein and glucokinase. J Bacteriol 176: 2133–2135.
32 Stephanopoulos G and JJ Vallino. 1991. Network rigidity and metabolic engineering in metabolite overproduction. Science 252: 1675–1681.
33 Taguchi M, K Izui and H Katsuki. 1977. Activation of *Escherichia coli* phosphoenolpyruvate carboxylase by guanosine-5′-diphosphate-3′-diphosphate. FEBS Lett 77: 270–272.
34 Utter MF and HM Kolenbrander. 1972. Formation of oxaloacetate by CO_2 fixation of phosphoenolpyruvate In: The Enzymes, vol VI (Boyer PD, ed), pp 117–168, Academic Press, New York.
35 Viikari L. 1988. Carbohydrate metabolism in *Zymomonas*. CRC Crit Rev Biotech 7: 237–261.
36 Viikari L and M Korhola. 1986. Fructose metabolism in *Zymomonas mobilis*. Appl Microbiol Biotechnol 24: 471–496.
37 Wächtershäuser G. 1990. Evolution of the first metabolic cycles. Proc Natl Acad Sci USA 87: 200–204.