Chapter 1

Glucose metabolism in actinomycetes

A.M.C.R. Alves
1. General aspects of actinomycetes

Actinomycetes are Gram-positive bacteria belonging to the order Actinomycetales (Lechevalier and Lechevalier, 1981), characterized by the formation of substrate and aerial mycelium on solid media, presence of spores and a high GC content of the DNA (60-70 mol%). The composition of the growth media can profoundly affect the growth and stability of substrate and aerial mycelium (Kalakoutskii and Agre, 1976). The majority of actinomycetes are soil bacteria that play an important role in mineralization processes in nature. Actinomycetes form a very important class of bacteria since they produce numerous natural products such as antibiotics and enzymes (Edwards, 1993). More than 50% of the known natural antibiotics produced are from actinomycetes (Berdy, 1984; Miyadoh, 1993). These bacteria can be separated into different genera on the basis of morphological, physical and chemical criteria. Actinomycetes possess cell wall type I to IV, depending on the presence of L-diaminopimelic acid (DAP) and glycine (type I), meso-DAP and glycine (type II), meso-DAP (type III), or meso-DAP, arabinose and galactose (Type IV) (Goodfellow, 1989).

*Streptomyces coelicolor* A3(2) is an actinomycete with cell wall type I, belonging to the family Streptomycetaceae (Stackebrandt and Woese, 1981; Goodfellow et al., 1992). It is one of the best studied *Streptomyces* species at the genetical level with a well characterized chromosomal physical map, carrying more than 150 genes or gene clusters (Hopwood et al., 1985, 1995; Kieser et al., 1992; Chater and Hopwood, 1993). Like other members of the order Actinomycetales, it has a complex life cycle involving three stages of differentiation. When aerial hyphae appear and sporulation occurs, the production of secondary metabolites is induced (Hopwood, 1988). It is thought that the morphological and physiological differentiation and the onset of secondary metabolite production result from common elements of regulation (Takano et al., 1992; Bibb, 1996). Studies have shown that mutants unable to sporulate (bald mutants) are also defective in regulation of production secondary metabolites (Chater and Hopwood, 1989; Chater and Bibb, 1996; Pope et al., 1996). *S. coelicolor* A3(2) produces at least four secondary metabolites: actinorhodin, undecylprodigiosin, A-factor and methylenomycin (Chater and Hopwood, 1989). Actinorhodin is a blue-pigmented dimeric isochromanequinone which is synthesized from an acetyl-CoA starter unit and seven malonyl-CoA extender units (Allman et al., 1981; Strohl and Connors, 1992). It is one of the best studied antibiotics with respect to its biosynthetic pathway and to its pleiotropic regulation (Allman et al., 1981; Horinouchi and Beppu, 1984; Malpartida and Hopwood, 1984; Doull and Vining, 1990; Hutchinson et al., 1993; Fernandez-Moreno et al., 1994).

*Amycolatopsis methanolica* belongs to the family of Pseudonocardiaceae (cell wall type IV) (Embley et al., 1988; Embley and Stackebrandt, 1994). It is one of the few methanol-utilizing Gram-positive bacteria, employing the ribulose monophosphate
(RuMP) cycle of formaldehyde fixation (fructose bisphosphate aldolase cleavage variant) (Hazeu et al., 1983). Because of its characteristics, this organism is a good candidate for fermentative production of aromatic amino acids and secondary metabolites derived from them (Dijkhuizen et al., 1985; de Boer, 1990). In recent years the main studies with this organism concerned the detailed characterization of the pathways involved in aromatic amino acid biosynthesis, methanol utilization, and the development of suitable plasmid vectors and of transformation systems (Dijkhuizen et al., 1993; Euverink, 1995; Vrijbloed, 1996).

Secondary metabolites are synthesized from primary metabolite precursors. Studies of primary metabolism in these type of organisms have been very limited but would provide important information about the switch between primary and secondary metabolism and for further improvement of industrial processes for the production of secondary metabolites.

2. Secondary metabolites are formed by different rearrangements from basic precursors of primary metabolism

Among Bacteria, secondary metabolites are produced by a restricted group: spore-forming bacteria, e.g. bacilli and actinomycetes (Zähner and Maas, 1972). During the normal life cycle of these organisms, sporulation occurs when their growth is impaired by the supply of oxygen, or nutrients, or by other environmental factors. It is at this point of the life cycle that secondary metabolites start to be produced (Malik, 1980; Martin and Demain, 1980).

A characteristic feature of secondary metabolism is that any given organism usually produces a group of compounds belonging to the same class (Kurylowicz et al., 1976). Normally these are relatively low molecular weight compounds (Maplestone et al., 1992; Bevan et al., 1995). Another feature of this type of metabolism is that a large number of products arises from relatively few intermediates in primary metabolism (Fig. 1). Also combinations of different primary metabolites are often used, e.g. in case of erythromycin where parts of the molecule are derived from propionate, glucose, and from methyl groups (Zähner and Maas, 1972; Zähner and Anke, 1983). In contrast to primary metabolites, secondary metabolites are not essential for growth (Vining, 1992). Primary metabolites are either building blocks for macromolecules, intermediates in reactions generating energy-rich compounds (ATP), coenzymes and vitamins. Secondary metabolites have no such vital roles in metabolism, but still may play an important role in the life cycle of the organism (Beppu, 1992). When the organism stops growing and enters a resting phase, accumulation of primary metabolites could occur. This is potentially harmful and it has
been speculated that the cells avoid this by starting to produce secondary metabolites (Malik, 1980; Vining, 1992).

The biosynthesis of secondary metabolites involves the following steps:

- Uptake of nutrients into the cell and conversion into intermediates of central metabolism.
- Accumulation of primary metabolites and signalling molecules induces secondary metabolite production.
- A branching-off of primary metabolites into the pathway peculiar for a specific antibiotic. Several primary metabolic pathways have been identified as sources of precursors for synthesis of some secondary metabolites (Fig. 1). These are: fatty acid metabolism (acetate and propionate for e.g. polyketide biosynthesis), amino acid metabolism (e.g. serine, cysteine, valine, tyrosine), carbohydrate metabolism (hexose phosphates, phosphoglycerate, phosphoenolpyruvate, pyruvate), purine and pyrimidine metabolism (adenine mononucleotide phosphate, AMP)(Katz and Demain, 1977; Martin and Demain, 1980; Laakel et al., 1994; Bevan et al., 1995; Herrmann, 1995; Revill et al., 1995).
- The production of these secondary metabolites is regulated by pathway-specific regulatory genes that determine the onset of antibiotic production.

3. Glucose metabolism and general pathways

3.1. Sugar metabolism in actinomycetes

Although actinomycetes are able to utilize a large number of sugars for growth, until recent years the knowledge concerning the pathways of carbohydrate metabolism and their regulation was still limited (Sabater et al., 1972a, b; Angell et al., 1992; White et al., 1992; Bramwell et al., 1993). During primary metabolism, the metabolic pathways involved are generally accurately tuned, to ensure the highest possible growth rate and maximum efficiency of growth under a given set of environmental conditions. This balanced growth is made possible by the involvement of a set of regulatory mechanisms, usually controlling a number of key enzymes at the levels of both their activity and their synthesis. For example the rate of glucose utilization may be controlled at the level of glucose transport (e.g. the glucose-PTS system), glucose phosphorylation (glucose kinase) and at other steps in glycolysis, e.g. at the conversion of fructose-6-phosphate (F-6-P) to fructose-1,6-bisphosphate (F-1,6-P2) by the enzyme phosphofructokinase or the conversion of phosphoenolpyruvate (PEP) plus ADP into pyruvate plus ATP by the...
Figure 1. Relationship between some precursors of primary metabolism and antibiotic production. Numbers in italic indicate the enzymes catalysing each glycolytic step. 1, hexokinase; 2, glucose-6-phosphate isomerase; 3, phosphofructokinase; 4, fructose-1,6-bisphosphate aldolase; 5, triose phosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7, phosphoglycerate kinase; 8, phosphoglycerate mutase; 9, enolase; 10, pyruvate kinase. Modified after Malik (1980). Solid arrows represent reactions of primary metabolism. Dashed arrows represent pathways for several groups of secondary metabolites.
enzyme pyruvate kinase. In the two actinomycetes *S. coelicolor* A3(2) and *A. methanolica*, subject of study in this thesis, the pentose phosphate pathway and the Embden-Meyerhof-Parnas (Glycolysis) pathway are the major pathways of glucose metabolism (chapters 2 and 4). The Entner-Doudoroff pathway additionally observed in *Escherichia coli* (Fraenkel, 1996), involving the reactions catalysed by the enzymes 6-phosphogluconate dehydrase (EC 4.1.12) and 2-keto-3-deoxy-6-phosphogluconate aldolase (EC 4.1.2.14), was not detected (Fig. 2).

### 3.1.1. Glucose uptake and the PTS system

In many bacteria sugars enter the cell via an inducible or constitutive uptake system. The phosphoenolpyruvate: sugar phosphotransferase system (PTS) is known to function in carbohydrate transport in bacteria (Reizer *et al.*, 1994; Saier *et al.*, 1995, 1996). More than 20 different carbohydrates are transported via this system which exclusively uses phosphoenolpyruvate (PEP) as phosphoryl donor in a phosphoryl transfer chain that involves the two energy-coupling proteins, Enzyme I and HPr, as well as the sugar specific, membrane-bound Enzyme II complexes. The Enzyme II permease translocates the sugar substrates into the cytoplasmic compartment of the bacteria concomitant with phosphorylation (Saier and Reizer, 1992; Postma *et al.*, 1993). Yet, actinomycete cells grown on sugars such as glucose or fructose show glucose and fructose kinase activity, suggesting that the transport of these sugars is not of the PTS type (Sabater *et al.*, 1972a; Ikeda *et al.*, 1984; Angell *et al.*, 1992). Glucose kinase negative mutants have been isolated and indeed were no longer able to grow on glucose (Hodgson, 1982) (chapters 2 and 5).

A glucose-PTS system also has been detected in the high-GC Gram-positive bacterium *Corynebacterium glutamicum* (Malin and Bourd, 1991). However, in 3 *Streptomyces* species, *S. coelicolor* A3(2), *Streptomyces lividans* TK23 and *Streptomyces griseofuscus* C581, only parts of a fructose-specific PTS system have been identified, but their physiological role remains to be determined (Titgemeyer *et al.*, 1995).

### 3.1.2. Glucose repression

The apparent lack of a PTS system for glucose uptake in actinomycetes (Titgemeyer *et al.*, 1995) also suggests that the mechanism of glucose repression in these organisms is very different from that in for instance *E. coli* (Angell *et al.*, 1992). In *S. coelicolor* A3(2), glucose represses the expression of many genes involved in the utilization of alternative carbon sources (e.g. arabinose, glycerol) (Hodgson, 1982; Smith and Chater, 1988; Delic *et al.*, 1992; Angell *et al.*, 1994; Hindle and Smith, 1994). In this case glucose kinase may mediate glucose repression via the synthesis of a metabolite that acts as a
Pathways for glucose metabolism. Grey arrows indicate the enzymes of the Entner-Doudoroff pathway. Dashed arrows indicate enzymes of the pentose phosphate pathway and black arrows indicate enzymes of the glycolytic pathway. 1, hexokinase; 2, glucose-6-phosphate (G6P) isomerase; 3, phosphofructokinase; 4, fructose-1,6-bisphosphate (F1,6P2) aldolase; 5, triosephosphate isomerase; 6, 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase; 7, 6-phosphogluconate (6PG) dehydrase; 8, glucose-6-phosphate dehydrogenase; 9, 6-phosphogluconate dehydrogenase; 10, ribose-5-phosphate (Ri5P) isomerase; 11, ribulose-5-phosphate (Ru5P) epimerase; TK, transketolase; TA, transaldolase; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; X5P, xylulose-5-phosphate.
repressing signal, with glucose-6-phosphate being the best candidate. Alternatively, glucose kinase may function more directly in mediating glucose repression through interaction or modification of a regulatory protein that interacts with the promoter regions of glucose repressible genes. It is also conceivable that the glycolytic flux increases when glucose is phosphorylated and that this is an important signal in the glucose repression pathway (Angell et al., 1992).

4. The Embden-Meyerhof-Parnas Pathway (Glycolysis): Enzymes and main points of regulation

Glycolysis is the central pathway of carbohydrate metabolism present in almost all cells (Fothergill-Gilmore and Michels, 1993). It is one of the most primitive pathways for sugar degradation since enzymes belonging to this route (or to modified ones) were also shown to be present in Archaea such as Thermoproteus tenax (Siebers and Hensel, 1993) and in the hyperthermophilic anaerobic bacterium Thermotoga maritima (Schröder et al., 1994). A modified pathway with ADP-linked kinases was found in the hyperthermophilic archaeon Pyrococcus furiosus (Kengen et al., 1994, 1996). Glycolysis serves various functions in cellular metabolism: During the breakdown of sugar molecules the favourable free energy of some reactions is harnessed to drive other cellular processes. Reducing equivalents are made available to the cell and building blocks are provided for the synthesis of for instance amino acids, fatty acids and sterols. The glycolytic pathway also shares some enzymes with other metabolic systems such as gluconeogenesis, the pentose phosphate pathway and the Calvin cycle (Fructose 1,6-bisphosphate aldolase, phosphofructokinase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triose phosphate isomerase). In order to control these reactions and to adjust the glycolytic flux to the cellular needs for energy and for precursors of biosynthetic pathways, a number of control mechanisms have evolved.

Two major control points have been identified, situated at the level of the enzymes that catalyse irreversible steps: phosphofructokinase and pyruvate kinase (Hofmann, 1976; Uyeda, 1979; Fraenkel, 1996). The activity of these enzymes can be allosterically controlled by different glycolytic intermediates and also by other metabolites. However, there is growing evidence that the regulation of glycolysis in actinomycetes differs in some steps from the conventional pathway present in other organisms (this thesis).
Hexokinase (EC 2.7.1.1) catalyses the first step in sugar utilization (in the absence of a PTS system) by transfer of a phosphor group from ATP or other phosphor donors (GTP, polyphosphates (PolyPn)) to sugars, e.g. a glucose unit. Under physiological conditions, the reaction is essentially irreversible. In general, mammals and yeast hexokinases exist as different isoenzymes (Fothergill-Gilmore and Michels, 1993). Normally they can be active as monomers or dimers. The mammalian hexokinases have subunit molecular masses of 100 kDa whereas the yeast hexokinases have a subunit size of 50 to 55 kDa (van Schaftingen et al., 1994). The best characterized hexokinases are those of the yeast Saccharomyces cerevisiae (Stachelek et al., 1986). The major regulator of mammalian hexokinase is the reaction product glucose-6-phosphate (G-6-P), inhibiting enzyme activity. Normally bacterial enzymes are smaller, with a subunit molecular mass of 30 to 35 kDa. In the case of Zymomonas mobilis, two distinct hexokinases have been characterized, a glucose and a fructose kinase (Scopes et al., 1985). The glucose kinase activity is inhibited by G-6-P as in the case of mammalian enzymes. Fructose kinase activity is inhibited by glucose, while fructose does not show any effect on glucose kinase activity (Zembrzuski et al., 1992).

In the actinomycete Actinomyces naeslundii a Poly(Pn)/ATP/GTP glucose kinase has been detected (Takahashi et al., 1995). Also in organisms such as Mycobacterium tuberculosis (Hsieh et al., 1993) and Propionibacterium shermanii (Wood and Goss, 1985), Poly(Pn)/ATP glucose kinases have been characterized. It has been speculated that glucose phosphorylation was originally mediated by polyphosphates and when ATP became available in the environment a transition took place. This “bifunctional” glucose kinase thus could represent an intermediate in the evolution of these enzymes (Hsieh et al., 1996b).

Recently, a new ADP-dependent glucose kinase has been detected in the archaeon P. furiosus (Kengen et al., 1995). This enzyme is a dimer as observed for glucose kinase enzymes from bacterial sources. In the actinomycete S. coelicolor A3(2), the gene coding for glucose kinase enzyme (glk) has been cloned (Angell et al., 1992). The glk gene restored glucose kinase activity in glucose kinase mutants of S. coelicolor A3(2) (Angell et al., 1994).
4.2. Glucose phosphate isomerase

The second step in glycolysis, the isomerization of glucose 6-phosphate to fructose 6-phosphate, is catalysed by the enzyme glucose phosphate isomerase (EC 5.3.1.9). Normally this enzyme is present as a dimer of Mr 66,000 (Fothergill-Gilmore and Michels, 1993). In the case of the mouse enzyme it also contains neurotrophic activity (Gurney et al., 1986). No information is available about the enzyme in actinomycetes.

4.3. Phosphofructokinase

4.3.1 ATP-dependent Phosphofructokinase

In most organisms, phosphofructokinase is a key regulatory enzyme of the glycolytic pathway. It catalyses the irreversible ATP-dependent phosphorylation of fructose-6-phosphate (EC 2.7.1.11). This enzyme requires Mg\(^{2+}\) since MgATP is its true substrate of the enzyme. The substrate ATP can be replaced by other nucleoside triphosphates such as ITP, GTP, UTP or CTP. F-6-P can be replaced by sedoheptulose-7-phosphate, fructose-1-phosphate, tagatose-6-phosphate or glucose-1-phosphate (Hofmann, 1976; Uyeda, 1979; Lobo et al., 1991). The ATP-dependent phosphofructokinases (ATP-PFKs) are in many cases allosterically regulated, have a high \(K_m\) for their substrate fructose-6-phosphate, and a neutral pH optimum (Hofmann, 1976; Uyeda, 1979; Fothergill-Gilmore and Michels, 1993).

ATP-PFKs can be classified in three groups according to their molecular masses (Evans et al., 1981):

**Group 1** - The bacterial enzyme has normally a tetrameric structure of identical subunits each with a molecular mass of 35 kDa and is allosterically regulated by phosphoenolpyruvate and ADP. However, there are examples of PFKs with a tetrameric
structure without allosteric regulation, as is the case in *Lactococcus lactis* (Llanos et al., 1993). One of the most well-characterized ATP-PFK from bacterial sources is the PFK1 enzyme from *E. coli* (Kotzlar and Buc, 1977; Buschmier, 1985; le Bras and Garel, 1985; Teschner et al., 1990; Johnson and Reinhart, 1992). This PFK1 is the major form of PFK in *E. coli* and accounts for 90% of the total PFK activity found in extracts. The remaining 10% is due to a second PFK (PFK2) (Kotzlar and Buc, 1981; Guixe and Babul, 1985). The genes for PFK1 (*pfkA*) and for PFK2 (*pfkB*) have been cloned and sequenced (Daldal, 1983; Hellinga and Evans, 1985), but no clear separate physiological roles for these two enzymes have been established (Daldal et al., 1982). In fact, deletion of the *pfkB* gene did not give a clear difference in growth or metabolic levels of different intermediates of glycolysis (Torres and Babul, 1991). The PFK1 enzyme from *E. coli* and the PFK enzyme from *Bacillus stearothermophilus* are the best studied enzymes with respect to kinetics and allosteric regulation (Blangy et al., 1968; Lau et al., 1987).

The steady-state kinetics of the *E. coli* PFK1 enzyme is consistent with a concerted allosteric mechanism in which two conformational states of the protein R and T are in equilibrium. The R and T states of PFK1 have the same affinity for ATP but the affinity of the R state for the substrate F-6-P is 2000 times higher (Bonne and Garel, 1992). The effectors ADP or GDP (activators of the PFK enzyme activity) bind preferentially to the R state and PEP (inhibitor of the PFK enzyme activity) binds to the T state.

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The crystal structure of the *E. coli* PFK1 enzyme has been determined (Shirakihara and Evans, 1988; Scrimmer and Evans, 1990; Kundratt and Evans, 1991). Each of the 4 PFK subunits is divided (Fothergill-Gilmore and Michels, 1993) into two domains. Each active site is in a cleft between two domains and each effector site lies between the two subunits. It has been proposed that this enzyme (as well as other bacterial PFKs) has four active sites and four MgADP effector sites per tetramer. This proposal is substantiated by analysis of the amino acid residues involved in ligand binding and catalysis (Shirakihara and Evans, 1988). The most important catalytic groups identified for the *E. coli* PFK1 enzyme are Asp-127, Asp-103, Asp-129, Thr-125, Arg-72 and Arg-171. Arg-162 and Arg-243 are the major contributors for the binding of F-6-P (Shirakihara and Evans, 1988; Kundratt and Evans, 1991). During the reaction catalysed by PFK, the phosphoryl group transferred from ATP interacts with Thr-125. The replacement of the Thr-125 by Ser changes the saturation by F-6-P from cooperative to hyperbolic, and abolishes the allosteric inhibition by PEP (Auzat et al., 1994b). Instead of distinct sites for allosteric activation and inhibition, PFK1 has only a single regulatory site, clearly separated from the active site (Lau et al., 1987; Lau and Fersht, 1989). Mutations in residues Arg-21, Arg-25 and Lys-213 make the enzyme insensitive or less sensitive to both activation by GDP and inhibition by PEP, confirming that these ligands bind at the same site (Shirakihara and Evans, 1988).
In addition to these residues, allosteric regulation involves also some residues located “between” interacting sites; these residues constitute the structural connection between the active and the regulatory sites. The residue Leu-178, does not belong to the regulatory or active site, but it is crucial for transmitting the conformational changes responsible for the heterotropic interactions in the *E. coli* PFK1 (Serre et al., 1990). Also residue Glu-187 (*B. stearothermophilus* numbering) (Lau and Fersht, 1989), is crucial in triggering the allosteric transition. In the *E. coli* enzyme a change of the Glu-187 residue into Asp induces only a minor conformational change whereas a neutral residue mutation Glu-187 to Asn decreases the cooperativity of F-6-P in the presence of PEP (Lau et al., 1987; Lau and Fersht, 1989; Auzat et al., 1994a).

ATP-PFK enzymes also have been detected in actinomycetes and are described in this thesis (chapters 4 and 5).

**Group 2** - Enzymes of this group are rather similar to enzymes of group 1 but their subunit sizes are much larger. These enzymes are present in mammals and are composed of four identical subunits of 85 kDa (Cai et al., 1990; Fothergill-Gilmore and Michels, 1993). Enzymes of this group have a greater number of effectors regulating their activity e.g. fructose-2,6-bisphosphate, citrate, ATP and 2,3-diphosphoglycerate (Hofmann, 1976).

**Group 3** - Enzymes of this group are octamers (αβ)4 composed of two nonidentical subunits with molecular masses of 112 and 118 kDa (Kriegel et al., 1991) present in yeasts. Sequence comparisons among the three classes of ATP-PFK proteins indicate that the yeast and mammalian enzymes have arisen as a result of gene duplication and fusion events (Heinisch et al., 1989; Fothergill-Gilmore and Michels, 1993). The activities of the yeast and mammalian enzymes are generally regulated by citrate, ATP and fructose-2,6-bisphosphate (F-2,6-P2). This last metabolite has been shown to play an important role in carbohydrate metabolism in yeast cells and cells of higher organisms (Hue and Rider, 1987; Boles et al., 1996). F-2,6-P2 activates 6-phosphofructokinase and strongly inhibits fructose-1,6-bisphosphate-1-phosphohydrolase (F-1,6bPase) increasing in this way the glycolytic flux. F-2,6-P2 is formed from fructose-6-phosphate and ATP by the enzyme 6-phosphofructo-2-kinase (6PF-2-K; EC 2.7.1.105). In mammalian cells, 6PF-2-K also catalyses degradation of F-2,6-P2 to form F-6-P and inorganic phosphate by a specific fructose-2-6-bisphosphate-2-hydrolase activity (Boles et al., 1996). In the case of the yeast *S. cerevisiae*, separate enzymes synthesize and degrade F-2,6-P2. In liver and in plants, the level of F-2,6-P2 is a main control point of regulation in carbohydrate metabolism (Hue and Rider, 1987; Sobrino et al., 1987; Paul and Stitt, 1993). However, in yeast the physiological importance of F-2,6-P2 is not yet clear (Nissler et al., 1984; Boles et al., 1993, 1996).
4.3.2 PP\textsubscript{i}-dependent Phosphofructokinase

An alternative rare form of PFK that uses inorganic pyrophosphate (PP\textsubscript{i}) as phosphor donor (PP\textsubscript{i}-PFK enzyme, EC 2.7.1.90). It catalyses a readily reversible reaction; it can replace both ATP-PFK and fructose-1,6-bisphosphatase. This enzyme has been discovered for the first time in the parasitic amoeba *Entamoeba histolytica* (Reeves et al., 1974). Two types of PP\textsubscript{i}-PFK are known: The activity of type 1, which is a homodimer, homotetramer or monomer is independent of F-2,6-P\textsubscript{2}. Type 1 does not coexist with ATP-PFK and is found in parasitic protists such as *Eimeria tenella* (Denton et al., 1994), *Giardia lamblia* (Philips and Li, 1995), *Trichomonas vaginalis* (Mertens et al., 1989) and in anaerobic bacteria such as *P. shermanii* (O'Brien et al., 1975), the hyperthermophilic bacterium *Spirochaeta thermophila* (Janssen and Morgan, 1992) and the hyperthermophilic archaeon *T. tenax* (Siebers and Hensel, 1993). Type 2 PP\textsubscript{i}-PFK is stimulated by F-2,6-P\textsubscript{2}, coexists with ATP-PFK and has a molecular mass larger than that of type 1 PP\textsubscript{i}-PFK. It is found exclusively in photosynthetic organisms, higher plants and the non-photosynthetic protist *Euglena gracilis* (Balogh et al., 1984; Enomoto et al., 1991).

Also several actinomycetes (Alves et al., 1994; Seiler et al., 1996) (chapter 2) were found to possess a type 1 PP\textsubscript{i}-PFK.

A correlation between anaerobic metabolism and the presence of PP\textsubscript{i}-PFK has led in the past to the suggestion that this enzyme is better adapted to anaerobiosis than its ATP-dependent counterpart (Mertens, 1991). Adenosine triphosphate (ATP) can be used to drive biochemical reactions. PP\textsubscript{i} can also be used directly as a source of high-energy phosphate.

4.3.2.1. Sources of PP\textsubscript{i} and its energetic advantage

The use of PP\textsubscript{i}-PFK permits utilization of PP\textsubscript{i} that is formed in many metabolic reactions like the biosynthesis and degradation of nucleic acids (DNA and RNA metabolism), proteins (Dawes and Senior, 1973; Kulaev and Vagabov, 1983) and polysaccharides (glycogen biosynthesis) (Takahashi et al., 1995), rather than a loss through wasteful removal by hydrolysis (Kulaev and Vagabov, 1983; Mertens, 1991). On this basis replacement of ATP-PFK by PP\textsubscript{i}-PFK could theoretically improve the net ATP yield of glucose degradation. This energetic advantage would be important in the...
case of fermentative metabolism. The energetic advantage discussed here rests on the assumption that the PP$_i$ used is a by-product of the biosynthesis of macromolecules. To allow such biosynthetic reactions to occur the concentration of PP$_i$ must be kept at a low level. This can be achieved by hydrolysis of PP$_i$ by the pyrophosphatase enzyme (Chen et al., 1994) or by other PP$_i$-consuming enzymes such as the PP$_i$-PFK enzyme (Table 1). Interestingly, organisms possessing a PP$_i$-PFK activity contain a very low level of pyrophosphatase activity (E. histolytica (Reeves et al., 1974), G. lamblia (Li and Philips, 1995), T. vaginalis (Mertens et al., 1989), A. naeslundii (Takahashi et al., 1995), P. freudenreichii (O’Brien et al., 1975).

Another point to be discussed concerns possible alternative sources of PP$_i$. It may be generated as a result of metabolic cycling between glycogen and glucose-1-P through the action of UDPG pyrophosphorylase, glycogen synthase and glycogen pyrophosphorylase (NDP-glucose synthase: NTP + glucose-1-P $\rightarrow$ NDP-glucose + PP$_i$) (Fig. 3).

Another possibility has been proposed for the organisms P. freudenreichii and E. histolytica, which in addition to the PP$_i$-PFK activity possess other unusual PP$_i$-dependent enzymes (Mertens, 1993) (Table 1). These are phosphoenolpyruvate carboxytransphosphorylase (EC 4.1.1.38) and pyruvate orthophosphate dikinase (PPDK,
E.C. 2.7.9.1). In these examples the first reaction would provide PP\(_i\) and the second reaction, towards the formation of pyruvate, would require PP\(_i\) (Table 1). Finally, on the evolutionary point of view, PP\(_i\) is believed to be a more ancient source of energy than ATP (Keefe and Miller, 1995). The occurrence of a PP\(_i\)-dependent glycolysis in representatives of the oldest eukaryotic branches (G. lamblia, T. vaginalis, E. histolytica), might be taken as experimental support for this hypothesis. This has led to the suggestion that biochemical pathways such as glycolysis evolved from being PP\(_i\) driven to ATP driven (Kulaev and Vagabov, 1983; Wood, 1985).

3.3.2.2. Enzymes involved in synthesis and degradation of polyphosphate

Polyphosphate (PolyP\(_n\)) has been found in volcanic condensates, deep-oceanic steam vents and in many organisms. It has been considered as a “molecular fossil”. From some of the reactions involved in PolyP\(_n\) metabolism, PP\(_i\) is formed (Kornberg, 1995). The occurrence of a PP\(_i\)-dependent glycolysis in representatives of the oldest eukaryotic branches (G. lamblia, T. vaginalis, E. histolytica), might be taken as experimental support for this hypothesis. This has led to the suggestion that biochemical pathways such as glycolysis evolved from being PP\(_i\) driven to ATP driven (Kulaev and Vagabov, 1983; Wood, 1985).

Table 1. Pyrophosphate dependent reactions and the corresponding nucleotide-dependent reactions. Adapted from Mertens (1993).

<table>
<thead>
<tr>
<th>Classical NTP-linked reactions</th>
<th>Pyrophosphate-linked reactions</th>
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<tbody>
<tr>
<td>ATP + F-6-P → F-1,6-P(_2) + ADP</td>
<td>PP(_i) + F-6-P ↔ F-1,6-P(_2) + ADP</td>
</tr>
<tr>
<td>ATP-phosphofructokinase</td>
<td>PP(_i)-phosphofructokinase</td>
</tr>
<tr>
<td>PEP + ADP → Pyruvate + ATP</td>
<td>PEP + AMP + PP(_i) → Pyruvate + ATP + P(_i)</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>Pyruvate orthophosphate dikinase</td>
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<tr>
<td>PEP + GDP + CO(_2) ↔ Oxaloacetate + GTP</td>
<td>PEP + P(_i) + CO(_2) ↔ Oxaloacetate + PP(_i)</td>
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<tr>
<td>phosphoenolpyruvate carboxylase</td>
<td>phosphoenolpyruvate carboxytransphosphorylase</td>
</tr>
<tr>
<td>Acetyl-P + ADP ↔ Acetate + ATP</td>
<td>Acetyl-P + P(_i) ↔ Acetate + PP(_i)</td>
</tr>
<tr>
<td>Acetate kinase</td>
<td>PP-aceate kinase</td>
</tr>
</tbody>
</table>

4.3.2.2. Enzymes involved in synthesis and degradation of polyphosphate

Polyphosphate (PolyP\(_n\)) has been found in volcanic condensates, deep-oceanic steam vents and in many organisms. It has been considered as a “molecular fossil”. From some of the reactions involved in PolyP\(_n\) metabolism, PP\(_i\) is formed (Kornberg, 1995). Table 2 gives a survey of these enzyme reactions. In the bacteria P. freudenreichii and A. naeslundii possessing PP\(_i\)-linked glycolysis, large amounts of polyphosphate have been detected (Clark et al., 1986; Takahashi et al., 1995). Also in these bacteria polyphosphate glucose kinase activity was present. These findings suggest that polyphosphate can be utilized in these organisms as an intracellular reservoir of energy (Takahashi et al., 1995).
Recently, a third alternative form of phosphofructokinase has been detected. The hyperthermophilic archaeon, *P. furiosus* employs a ADP-PFK enzyme in its modified Embden-Meyerhof pathway involving ADP-dependent kinases (Kengen *et al.*, 1994, 1996). To explain the use of ADP instead of ATP in the PFK reaction it has been suggested that ADP is more thermostable than ATP, and that, when the ATP level is low (after a starvation period), the organism is still able to phosphorylate glucose using ADP.

### Table 2. Enzymes involved in biosynthesis and degradation of polyphosphates. Adapted from van Alebeek (1994).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC number</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degradation of polyphosphate (PolyP&lt;sub&gt;n&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyphosphate glucosekinase</td>
<td>2.7.1.63</td>
<td>Glucose + PolyP&lt;sub&gt;n&lt;/sub&gt; → G6P + PolyP&lt;sub&gt;n-1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Polyphosphate:AMP phosphotransferase</td>
<td></td>
<td>PolyP&lt;sub&gt;n&lt;/sub&gt; + AMP → PolyP&lt;sub&gt;n-1&lt;/sub&gt; + ADP</td>
</tr>
<tr>
<td>Endopolyphosphatase</td>
<td>3.6.1.10</td>
<td>PolyP&lt;sub&gt;n&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;O → PolyP&lt;sub&gt;n-x&lt;/sub&gt; + PP&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>Exopolyphosphatase</td>
<td>3.6.1.11</td>
<td>PolyP&lt;sub&gt;n&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;O → PolyP&lt;sub&gt;n-1&lt;/sub&gt; + P&lt;sub&gt;i&lt;/sub&gt;</td>
</tr>
<tr>
<td>Tripolyphosphatase</td>
<td>3.6.1.25</td>
<td>PPR&lt;sub&gt;i&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;O → PP&lt;sub&gt;i&lt;/sub&gt; + P&lt;sub&gt;i&lt;/sub&gt;</td>
</tr>
<tr>
<td>Pyrophosphatase</td>
<td>3.6.1.1</td>
<td>PP&lt;sub&gt;i&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;O → 2P&lt;sub&gt;i&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC number</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degradation of polyphosphate (PolyP&lt;sub&gt;n&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyphosphate dikinase</td>
<td>2.7.4.1</td>
<td>ATP + PolyP&lt;sub&gt;n&lt;/sub&gt; ↔ ADP + PolyP&lt;sub&gt;n+1&lt;/sub&gt;</td>
</tr>
<tr>
<td>1,3-Diphosphoglycerate:polyphosphate phosphotransferase</td>
<td>2.7.4.17</td>
<td>1,3-DPG + PolyP&lt;sub&gt;n&lt;/sub&gt; ↔ 3-PG + PolyP&lt;sub&gt;n+1&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

### 4.3.3. ADP-dependent phosphofructokinase

\[
\begin{align*}
\text{Fructose-6-phosphate} & \quad \text{ADP} \\
\text{AMP} & \quad \text{Fructose-1,6-bisphosphate}
\end{align*}
\]

Recently, a third alternative form of phosphofructokinase has been detected. The hyperthermophilic archaeon, *P. furiosus* employs a ADP-PFK enzyme in its modified Embden-Meyerhof pathway involving ADP-dependent kinases (Kengen *et al.*, 1994, 1996). To explain the use of ADP instead of ATP in the PFK reaction it has been suggested that ADP is more thermostable than ATP, and that, when the ATP level is low (after a starvation period), the organism is still able to phosphorylate glucose using ADP.
4.4. Fructose-1,6-bisphosphate aldolase

Aldolase (E.C 4.1.2.13) catalyses the reversible cleavage of fructose-1,6-bisphosphate to triose phosphate and dihydroxyacetone phosphate (von der Osten et al., 1989; Fothergill-Gilmore and Michels, 1993). Two different classes of aldolases can be distinguished based on the reaction mechanisms (Alefounder et al., 1989). Class 1 aldolases, but not class 2, form an intermediate with their substrate through a Schiff base. Class 2 aldolases instead require a divalent cation like Ca$^{2+}$, Fe$^{2+}$ or Zn$^{2+}$ to stabilize the enzyme-substrate complex. Bacterial enzymes have been characterized revealing examples of both class 1 and 2 proteins (Fothergill-Gilmore and Michels, 1993). Class 1 proteins are homotetrameric enzymes with a subunit Mr of 40,000, typical for Eukarya (Horecker et al., 1972). Bacterial representatives of class 1 enzymes have been found in E. coli (Stribling and Perham, 1973), Lactobacillus casei (London, 1974) and Staphylococcal species (Rudolph et al., 1992; Witke and Gotz, 1993). Class 2 aldolases are dimeric proteins with identical subunits of Mr 40,000. The aldolases from yeast, a second aldolase in E. coli (Alefounder et al., 1989) and C. glutamicum (von der Osten et al., 1989) belong to this class. Only the crystal structures of class 1 enzymes from several Eukarya have been determined (Fothergill-Gilmore and Michels, 1993). No information is available about this enzyme from actinomycetes.

4.5. Triosephosphate isomerase

The interconversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate is catalysed by the enzyme triose phosphate isomerase (TIM E.C 5.3.2.1) (Fothergill-Gilmore and Michels, 1993). The enzyme does not require any cofactor. In Bacteria and Eukarya analysed, TIM is a homodimeric protein with a subunit size of Mr 27,000 (Fothergill-Gilmore and Michels, 1993), but in the hyperthermophilic archaeon
Pyrococcus woesei and Methanothermus fervidus TIM it is a homotetramer (Kohloff et al., 1996). It has been speculated that the tetrameric aggregation is correlated with increased thermostability. At present, amino acid sequences of this enzyme are available from representatives of most phylogenetic groups (Fothergill-Gilmore and Michels, 1993). No information is available about this enzyme from actinomycetes, however.

4.6. Glyceraldehyde-phosphate dehydrogenase

The next step in the pathway, the phosphorylation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, is catalysed by the enzyme glyceraldehyde-phosphate dehydrogenase (GAPdh, E.C 1.2.1.12). This enzyme is a homotetramer with a subunit size of M, 34,000-38,000. Each of the subunits can bind NAD⁺ (cofactor) and the binding can occur cooperatively. The primary sequences of different GAPdh enzymes have been determined (Fothergill-Gilmore and Michels, 1993). This enzyme is the most highly conserved enzyme from the glycolysis and the best characterized one at the structural level (Fothergill-Gilmore and Michels, 1993). The enzymes from Eukarya and Bacteria are clearly homologous whereas the enzymes from Archae have been shown to be very different from those of the other organisms (Fabry and Hensel, 1988; Fabry et al., 1989; Zwickl et al., 1990; Prub et al., 1993). A different origin has been proposed for the latter type of GAPdh (Fothergill-Gilmore and Michels, 1993). In the actinomycete Streptomyces aureofaciens, the gene encoding GAPdh (gap) has been cloned and sequenced, showing 52% amino acid identity with bacterial and eukaryotic gap genes (Kormanec et al., 1995).
4.7. 3-Phosphoglycerate kinase

Phosphoglycerate kinase (PGK) catalyses the reversible conversion of 1,3-phosphoglycerate to 3-phosphoglycerate (EC 2.7.2.3). This enzyme is normally a monomer with a Mr of 44,000 (Scopes, 1973). A large number of full primary sequences of PGK are now available. The enzymes from Eukarya, Bacteria and Archaea are quite homologous (Fothergill-Gilmore and Michels, 1993). In the hyperthermophilic bacterium T. maritima PGK exists in two forms. A separate form with a subunit size of 43 kDa, and as a fusion protein (together with the TIM enzyme) with a subunit size of 70 kDa. This is a unique example of a bifunctional enzyme in the glycolytic pathway combining the catalysis of two nonconsecutive reactions (Schurig et al., 1995).

4.8. Phosphoglycerate mutase

Phosphoglycerate mutase (PGM, EC 5.4.2.1) catalyses the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. It comprises a family of enzymes which catalyse reactions involving the transfer of phosphor groups among the three carbon atoms of phosphoglycerates. There are two major groups of PGM enzymes with different kinetic properties (Fothergill-Gilmore and Michels, 1993). Group 1 type of PGM is dependent upon 2,3-diphosphoglycerate (2,3-DPG) for activity. It has been found in vertebrates, yeast (Fothergill-Gilmore and Watson, 1989) and also recently in two actinomycetes S. coelicolor A3(2) (White et al., 1992) and, in A. methanolica (Alves et al., 1994) (chapter 2). Cofactor-dependent PGMs are active as monomers, dimers or tetramers depending upon the organism from which they have been isolated. The cofactor-dependent PGM from S. cerevisiae is the best studied PGM enzyme. The 3D-structure of this tetrameric enzyme has been solved and the amino acid residues involved in the active site have been assigned (Winn et al., 1981; Fothergill-Gilmore and
Chapter 1

Watson, 1989). In *S. coelicolor* A3(2) PGM is a tetrameric enzyme and in *A. methanolica* PGM1 is a monomer and PGM2 is a dimer (chapters 2 and 6).

Group 2 PGM is independent of 2,3-DPG for its activity; it occurs in higher plants and invertebrates and is independent of any co-factors (Fothergill-Gilmore and Watson, 1989; Fothergill-Gilmore and Michels, 1993). *Bacillus spp.* have 2,3-DPG independent PGMs which require manganese for activity (Vazquez-Leyva and Setlow, 1994). The cofactor-independent PGMs are monomers of Mr 60,000 (Fothergill-Gilmore and Michels, 1993).

Another category of PGM is a closely related enzyme (DPG mutase/synthase EC 5.4.2.4/EC 3.1.3.13) which catalyses the synthesis of 2,3-DPG and plays a role in controlling haemoglobin oxygen affinity (Fothergill-Gilmore and Michels, 1993). This enzyme is considered to be an isoenzyme of the vertebrate glycolytic PGM because of sequence similarities. DPG mutase possesses a high level of monophosphoglycerate mutase activity in addition to 2,3-DPG synthase and phosphatase activity, and it is frequently designated as bisphosphoglycerate synthase (Fothergill-Gilmore and Watson, 1989).

4.9. Enolase

Enolase catalyses the dehydration of 2-phosphoglycerate to phosphoenolpyruvate (EC 4.2.1.11). Two classes of enolases exist (Fothergill-Gilmore and Michels, 1993; Green *et al.*, 1993). The first class is a dimer of Mr 45,000 and it requires Mg$^{2+}$ for the stability of the enzyme and for substrate binding; the second class has an octameric structure. The first class includes enzymes from Eukarya and *E. coli* (Pawluk *et al.*, 1986). Enolase isolated from the *Clostridium* genus appear to have an octameric structure (Green *et al.*, 1993). Enolase has been more extensively studied in Eukarya than in Bacteria. Enolase appears to have a variety of functions in addition to its role in glycolysis. In the obligatively fermentative Gram-negative bacterium *Z. mobilis*, enolase is the most abundant glycolytic enzyme and it has been suggested to play a role in metabolic flux control in this organism (Pawluk *et al.*, 1986). Until now no studies of this enzyme in actinomycetes, have been carried out.
4.10. Pyruvate kinase

Pyruvate kinase (PK, EC 2.7.1.40) is one of the major regulatory enzymes of the glycolytic pathway. It catalyses the last step of the glycolysis, the irreversible reaction from PEP to pyruvate (Kayne, 1973). In most bacteria this protein is a tetramer composed of identical subunits of 500 amino acid residues, although some different structures have been identified (Fothergill-Gilmore and Michels, 1993). Almost all PKs show positive cooperativity in binding the substrate PEP (Fothergill-Gilmore and Michels, 1993; Sakai and Ohta, 1993). In addition, they are often regulated by a number of allosteric effectors like glucose-6-phosphate, ribulose 5-phosphate, AMP and F-1,6-P$_2$ (le Bras and Garel, 1993). Two different mechanisms of regulation at the activity level can be found in Bacteria.

E. coli and Salmonella typhimurium each have two differently regulated isoenzymes (Fothergill-Gilmore and Michels, 1993; Ponce et al., 1995) with different kinetic properties. In E. coli the type 1 PK isoenzyme is constitutive and allosterically activated by F-1,6-P$_2$. This enzyme is also susceptible to a synergistic cooperative feedback inhibition by succinyl-CoA and ATP, and to inhibition by phosphate. The type 2 PK isoenzyme is inducible and stimulated by AMP and by some sugar monophosphates such as ribose-5-phosphate and glucose-6-phosphate (Ponce et al., 1995). The physiological roles of the two pyruvate kinases and their regulation may be as follows. When cells are grown on a glycolytic substrate, the intracellular concentration of F-1,6-P$_2$ is high, thus stimulating the activity of isoenzyme 1. Under conditions where gluconeogenesis is predominant, the F-1,6-P$_2$ concentration is at such a low level that the enzyme is virtually inactive, whatever the concentration of PEP. However, the PEP level is such that the isoenzyme 2 can catalyse the reaction at 30-40% of the maximal rate. A decrease of the cellular energy charge would then, via the effector AMP, activate the enzyme and increase the ATP concentration (Fothergill-Gilmore and Michels, 1993; Ponce et al., 1995).

In some protists and bacteria (E. histolytica, Bacteroides symbiosus, G. lamblia and P. freudenreichii) PK activity is absent and substituted by PPDK (Wood et al., 1977; Wood and Goss, 1985; Mertens, 1991). This enzyme differs in the use of the substrate PP$_i$ instead of ADP, and the fact that it catalyses both the forward and the reverse reactions. Like the PK enzyme, it also requires the ions K$^+$, NH$_4^+$ and Mg$^{2+}$ for its activity. This type of activity (PPDK) is associated with other pyrophosphate-linked reactions such as
**Organism** | **Gene Organization** | **Reference**
--- | --- | ---
Sulfolobus solfataricus | pgk pgap | Jones et al., 1995
Thermotoga maritima | pgk gap | Schulp et al., 1995
Escherichia coli | pgk abp cdh tpi gap pgk fda | Hellings and Evans, 1985; Pichersky and Hess, 1984; Alefunder and Perham, 1989
Zymomonas mobilis | gap pgk | Conway et al., 1987, 1988
Bacillus megaterium | gap pgk tpi | Schlapfer and Zuber, 1992
Corynebacterium glutamicum | gap pgk tpi ppc | Eikmanns, 1996; Scheide et al., 1993
Lactococcus lactis | pfk pk ilp | Liens, 1993
Lactobacillus bulgaricus | pfk pk | Branny et al., 1993
Bacillus subtilis | pgk gpi pgm eno | Vazquez-Leyva and Setlow, 1994
Bacillus stearothermophilus | gap pgk | Davies et al., 1991; Brantlet et al., 1989

**Figure 4.** Examples of the organization of glycolytic genes in various bacteria. Phosphoglycerate kinase (pgk), glyceraldehyde-3-phosphate dehydrogenase (gap), triose phosphate isomerase (tpi), phosphofructokinase (pfk), pyruvate kinase (pk), enolase (eno), phosphoglycerate mutase (pgm), fructose-1,6-bisphosphate aldolase (fda), lactate dehydrogenase (ldh), periplasmic sulphate-binding protein (sbp), CDP-diglycerate hydrolase (cdh), phosphoenolpyruvate carboxylase (ppc). White bars correspond to genes coding for non-glycolytic enzymes, that are part of a cluster of glycolytic genes. White bar with dashed lines correspond to a possible pgk gene in *B. subtilis* (Vazquez-Leyva and Setlow, 1994).
the PP\textsubscript{i}-PFK, PEP carboxytransphosphorylase and PP\textsubscript{i}-acetate kinase (EC 2.7.2.12) (Mertens, 1993) (see also section 4.3.2.1)

PK has also been characterized from different Gram-positive bacteria such as \textit{C. glutamicum} (Jetten \textit{et al.}, 1994), \textit{Brevibacterium flavum} (Ozaki and Shiio, 1969), and from the actinomycetes \textit{S. coelicolor} A3(2) (personal communication L.V. Bystrykh) and \textit{A. methanolica} (this thesis, chapter 2). In these organisms (except in \textit{S. coelicolor} A3(2): dimer) the PK enzyme is a tetramer, and it is regulated at the activity level being activated by AMP and inhibited by ATP. The \textit{C. glutamicum} enzyme differs in that it does not require Mg\textsuperscript{2+} ions for activity but Mn\textsuperscript{2+} or Co\textsuperscript{2+} ions (Jetten \textit{et al.}, 1994).

5. Organization of genes encoding glycolytic enzymes

Gene clusters encoding enzymes involved in glycolysis have been identified in several bacteria. In some cases these clusters contain genes that code for enzymes catalysing irreversible steps in glycolysis such as ATP-PFK and PK. Alternatively genes encoding enzymes catalysing consecutive steps, such as GAPdh and PGK, may cluster. Further variations occur as well (Fig. 4). However, these findings show that considerable diversity exists among bacteria with respect to the transcriptional organization of their glycolytic genes. Clustering of genes encoding glycolytic enzymes has not been reported for actinomycetes.

REFERENCES

References are listed on pages 129-140.