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Pyrophosphate-Dependent Phosphofructo-1-Kinase Complements Fructose 1,6-Bisphosphatase but Not Phosphofructokinase Deficiency in *Escherichia coli*

ROBERT G. KEMP* AND RAKESH L. TRIPATHI

Department of Biological Chemistry, University of Health Sciences, The Chicago Medical School, 3333 Green Bay Road, North Chicago, Illinois 60064

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The gene from *Propionibacterium freudenreichii* for PP_i-dependent phosphofructo-1-kinase, an enzyme that is found in some bacteria, in a number of anaerobic protists, and in plants, complements the absence of fructose 1,6-bisphosphatase in *Escherichia coli* but does not complement the deficiency of the ATP-dependent phosphofructokinase.

A number of prokaryotes and protists utilize a PP_i-dependent phosphofructo-1-kinase (PFK) in place of the familiar ATP-dependent enzyme that is broadly distributed in the biosphere. These organisms are anaerobic and do not contain fructose 1,6-bisphosphatase (FbPase), the enzyme critical for the reversal of glycolysis in other organisms. Higher plants contain both PP_i-dependent and ATP-dependent PFKs as well as a distinct FbPase. The three activities producing or degrading fructose 1,6-bisphosphate are summarized in Fig. 1. Mertens (7) has recently reviewed the properties of PP_i-dependent PFKs and has suggested that their role is that of a glycolytic enzyme adapted to anaerobic conditions. On the other hand, other investigators (3, 10) have pointed out the reversibility of the PP_i-dependent PFK and have suggested that, at least in plants, it may function in the reverse direction, that is, as an FbPase. The overall free energy change for reaction 1 in Fig. 1 is about 2 kcal (~ 8 kJ) less than that for the ATP-dependent enzyme (reaction 2), a fact that permits the consideration of reversibility (7). Furthermore, if the ratio of P_i to PP_i is maintained at a high level by a pyrophosphatase, the reverse reaction of the PP_i-dependent enzyme would be favored.

Our laboratory has recently cloned and expressed in *Escherichia coli* the nonallosteric PP_i-dependent PFK from *Propionibacterium freudenreichii* (5). The availability of an expression system allows us to examine whether the PP_i-dependent PFK is capable of complementing a deficiency in *E. coli* of either the ATP-dependent PFK or FbPase. *E. coli* contains two ATP-dependent PFKs, an allosteric enzyme which accounts for about 90% of the total PFK activity and a nonallosteric PFK that is structurally unrelated to the major PFK. Fraenkel's laboratory has produced an *E. coli* strain (11) in which both PFK genes have been deleted: DF1020 [*pro-82 pfkB201 recA56 endA1* (*rha-pfkA*)*200 hsdR17 supE44*]. This strain is incapable of growth on minimal media supplemented with mannitol. In the current study, this strain was transformed with a plasmid containing the gene for PP_i-dependent PFK to determine whether the PP_i-dependent enzyme would allow growth on mannitol as an energy source. Fraenkel's laboratory has produced also an *E. coli* strain from which the gene for FbPase has been deleted (9). This strain, designated DF657 [*tonA22 ompF627* (*T2R*) *relA1 pit-10 spoT1* (*fbp*)*287*] cannot grow on minimal medium containing glycerol and succinate as carbon sources. In addition, it was shown that the Fbp phenotype could be screened on MacConkey-glycerol indicator plates (9). Fbp^+ strains form red colonies, whereas Fbp^- strains form pale, yellow colonies.

Table 1 shows the results of attempts to complement PFK in the strain lacking PFK, DF1020, and to complement FbPase in the strain lacking FbPase, DF657. As shown previously (1), DF1020 could grow on minimal medium supplemented with mannitol when transformed by a vector containing ATP-dependent PFK, by using a construct (pRZ3) containing *E. coli* PFK (11). In contrast to the results obtained with ATP-dependent PFK, no growth was seen in mannitol minimal medium when the transformation was carried out with pLG1, which consists of pBluescriptII into which has been inserted the gene for PP_i-dependent PFK from *P. freudenreichii* along with 160 bases upstream and 1,100 bases downstream of the coding sequence (5). On the other hand, FbPase-deficient bacteria transformed with a plasmid bearing PP_i-dependent PFK but not ATP-dependent PFK were capable of growing on succinate-glycerol medium and produced red colonies on MacConkey-glycerol indicator plates. IPTG (isopropyl-β-D-thiogalactopyranoside), which induces higher levels of PP_i-dependent PFK activity with pLG1 transformed cells, did not influence growth on minimal medium (data not shown).

The failure of PP_i-dependent PFK to permit growth on mannitol indicates that the reaction does not function efficiently in the forward direction, that is, toward formation of fructose bisphosphate, in *E. coli*. The most likely explanation for this failure is that the amounts of PP_i are too small. While PP_i is produced in a number of biosynthetic steps, it is

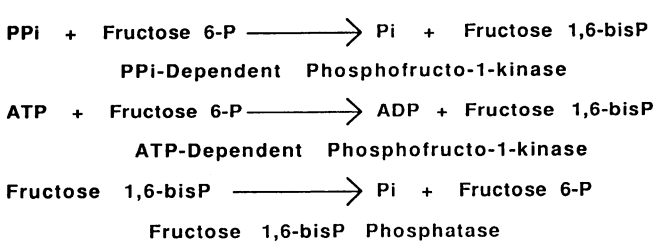

FIG. 1. Reactions involving fructose 1,6-bisphosphate.

* Corresponding author.

TABLE 1. Complementation of FbPase and PFK activities

Host strain and plasmid	Enzyme activity (U/g of cells)[a]			Growth on minimal medium with[b]:	
	PFK		FbPase	Mannitol	Glycerol + succinate
	ATP dependent	PP_i dependent			
DF1020					
pRZ3	17.6	<0.01		1.8 (2.7)	
pUC[c]	<0.01	<0.01		0.02 (—)[d]	
pLG1	<0.001	2.4		0.01 (—)	
DF567					
pRZ3	31	<0.01	<0.001		0.02 (—)
pLG1	1.5	3.7	3.5		1.5 (2.5)
pBluescriptII[e]	1.2	<0.01	<0.01		0.01 (—)

[a] PFK activity was determined with cells grown overnight on LB and assayed for ATP-dependent (11) or PP_i-dependent (5) activity. FbPase activity was determined in the standard assay (9) with the addition of 1 mM P_i. No activity was seen in dialyzed extracts when phosphate was not included.

[b] Growth was measured at A_{550} in minimal media at 30°C for DF1020 and 37°C for DF567. Maximum growth (in optical density units at A_{550}) in 2 days is given, with the doubling time (in hours) indicated in parentheses.

[c] pUC is the parent plasmid of pLG1.

[d] —, the rate of growth was too low for an accurate determination of doubling time.

[e] pBluescriptII is the parent plasmid of pLG1.

presumably maintained in very small amounts by pyrophosphatase, an enzyme present in most, if not all, organisms. While a systematic comparison of pyrophosphatases in organisms with either ATP- or PP_i-dependent PFKs has not been carried out, a few limited studies suggest that organisms with a PP_i-dependent enzyme may have very low pyrophosphatase activity. *Trichomonas vaginalis* and *Entamoeba histolytica* are two protists that have a PP_i-dependent PFK and that have no detectable metal ion-dependent pyrophosphatase and very little total pyrophosphatase activity (6, 8). On the other hand, *E. coli* has very high levels of pyrophosphatase activity but apparently continues to maintain a significant amount of PP_i (4). Kukko-Kalske et al. (4) have measured PP_i in exponentially growing *E. coli* cells and have reported concentrations of about 0.5 mM. This, one would presume, would be sufficient to permit phosphorylation of fructose 6-phosphate by the PP_i-dependent enzyme. On the other hand, the same investigators have reported that the PP_i concentration dropped dramatically to below the detection limit of the assay when glucose was exhausted from the medium. Danchin et al. (2) noted a rapid drop in the concentration of PP_i when the energy metabolism of *E. coli* was altered by the addition of 2-ketobutyrate, suggesting that a constant source of energy is necessary for the maintenance of PP_i levels. It might well be assumed that in the present experiments, the concentration of PP_i in *E. coli* in minimal medium in the presence of mannitol was never sufficient to permit glycolysis.

The data clearly demonstrate that the PP_i-dependent PFK can function as an FbPase both in minimal media and on the nutritionally more-complete MacConkey agar. One must assume that the concentration of P_i is very high relative to that of PP_i and that, in the absence of any other source of fructose 6-phosphate, reaction 1 (Fig. 1) will operate in the reverse direction. Under these conditions, the kinase, despite an unfavorable equilibrium constant, can perform the role of a gluconeogenic enzyme.

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