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Allosteric and non-allosteric E. coli phosphofructokinases: effects on growth

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Summary

In wild type Escherichia coli K-12 ca. 90% of phosphofructokinase is known to be the allosteric enzyme Pfk-1, and the rest is Pfk-2, a non-allosteric enzyme. An isogenic strain series has now been constructed with varying combinations and amounts of Pfk-1 and Pfk-2 (e.g., no Pfk-1, high level of Pfk-2; normal level Pfk-1, high level Pfk-2, etc.). In minimal medium with glucose, glucose-6-P, and glycerol, aerobically and anaerobically, provided there is adequate total amount of enzyme, what allosteric type it is does not make much difference to growth rate or yield of this organism.

Fructose-6-phosphate kinase (phosphofructokinase, E.C. 2.7.1.11) is a key enzyme of glycolysis which typically has complex kinetics and several effectors (1,2). It is implicit in many studies of this enzyme - including our own - that its allosteric properties are physiologically important. One way to assess how important would be to compare strains differing only in the allosteric properties of their phosphofructokinases.

The wild type strain of E. coli contains a well characterized allosteric phosphofructokinase, Pfk-1, which exhibits a sigmoid curve of velocity vs. fructose-6-P concentration, is inhibited by phosphoenolpyruvate and activated by nucleoside diphosphates (3). Pfk-1 is specified by the pfkA gene (4), and its amount is somewhat dependent on the cultural conditions, increasing two or three-fold in anaerobiosis (5-7).

The wild type strain also contains a low level (5-10% of the total activity) of a non-allosteric phosphofructokinase, Pfk-2 (8-10), specified by the gene pfkB (11). The amount of Pfk-2 varies little with growth

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conditions (6), but is greatly increased by the pfkB1 mutation (10). This mutation allows growth on sugars of mutants lacking Pfk-1 (12,13). Other mutations (e.g., pfkB1 pfkB2) result in complete loss of Pfk-2 activity (14).

We have now constructed an isogenic series of strains with various combinations of these alleles and, therefore, various amounts of the two different enzymes. It is not easy to anticipate the metabolic consequences. Perhaps an enzyme insensitive to normal inhibitors would function too quickly, and one insensitive to normal activators function too slowly. These differences might be reflected in abnormal growth rates or yields, unusual growth lags in transitions between different media, abnormal rates of metabolism and product formation by resting cells, changes in futile cycling, and so on. One might expect such differences with substrates such as glucose or glucose-6-P whose metabolsim needs phosphofructokinase, or even with a substrate such as glycerol which does not. (For example, in the latter case, a non-allosteric phosphofructokinase might act to reconvert fructose-6-P to fructose-1,6-P_2 too fast to maintain an adequate pool.)

Our studies of the first two parameters, growth rate and yield in minimal media with glucose, glucose-6-P, and glycerol, aerobically and anaerobically, show that these are not markedly affected by the type of phosphofructokinase.

Materials and Methods

Strains. A strain, RT142 (13), carrying a marker (metB) close to $pfkA^+$ and another (pps) near $pfkB^+$ was used as recipient in phage P1 transductions from strains with other alleles (pfkA1 and pfkB1 from strain DF77 (AM1R20 ref. 12), and pfkB1 pfkB2 from strain DF88 (ref. 14)) to make an isogenic series, DF531-DF536, with the pfk genes as indicated in Table 1. All the strains are, in addition, F^- edd galK his pyrD str sup ($metB^+$ pps^+). Phosphofructokinase activities (8), μmole/min/mg protein in crude extracts after growth in broth, were DF531, 0.28; DF532, 0.01; DF533, 0.78; DF534, 0.00; DF535, 1.21; DF536, 0.26.

Growth and yield. Growth was in minimal medium 63 (15) supplemented with histidine (25 μg/ml), uracil (50 μg/ml), and carbon source 2 mg/ml except for the glycerol (4 mg/ml) + fumarate (2 mg/ml) anoxic cultures. Anoxic media were prepared by bubbling medium with O_2-free N_2 immediately after autoclaving and stoppering the flasks when cool. A_{580} was measured with a Gilford Model 300 N spectrophotometer; samples from anoxic cultures were

Table 1. Growth and yield. The minimal media were inoculated 1:100 from minimal medium with glycerol (0.5 mg/ml). Air, aerobic incubation; N_2, incubation under N_2; dt, doubling time in min; Y, yield (μmoles substrate used/ml ΔA_{580}); NG, no growth; ND, not done.

		I		II		III		IV		V		VI	
		Glucose		Glucose-6-P		Glucose		Glucose-6-P		Glycerol		Glycerol + Fumarate	
		Air		Air		N_2		N_2		Air		N_2	
Strain	Enzyme	dt	Y	dt	Y	dt	Y	dt	Y	dt	Y	dt	Y
DF531 (*pfkA*$^+$ *pfkB*$^+$)	(Pfk-1 + low Pfk-2)	80	4.2	65	4.1	105	14.3	110	7.0	90	7.9	248	13.5
DF532 (*pfkA1* *pfkB*$^+$)	(only low Pfk-2)	900	ND	305	ND	NG		NG		100	ND	253	15.5
DF533 (*pfkA1* *pfkB1*)	(only high Pfk-2)	105	4.7	78	ND	135	14.5	245	17.1	100	7.9	243	14.3
DF534 (*pfkA1* *pfkB1* *pfkB2*)	(none)	NG		NG		NG		NG		100	ND	270	13.2
DF535 (*pfkA*$^+$ *pfkB1*)	(Pfk-1 + high Pfk-2)	80	6.0	65	4.6	105	11.0	100	9.9	95	7.9	270	14.1
DF536 (*pfkA*$^+$ *pfkB1* *pfkB2*)	(Pfk-1 only)	80	4.5	63	3.5	105	11.8	103	10.6	95	ND	305	ND

removed by syringe. Yields on glucose and glucose-6-P were determined in exponential growth by periodic assay of culture supernatants by glucose oxidase (Sigma) or glucose-6-P dehydrogenase (Boehringer), plotted vs. A_{580}; yields on glycerol were determined from stationary phase A_{580} in cultures with limiting glycerol.

Results and Discussion

The strain characteristics are easiest seen in Table 1, column I, aerobic growth on glucose. The wild type strain (line 1) grows well, the *pfkA* mutant (line 2), with only the low normal level of Pfk-2, grows very slowly, and the strain lacking both enzymes (line 4) does not grow at all. Strains carrying the high level of Pfk-2 grow, whether lacking Pfk-1 (line 3) or containing it (line 5). The strain with Pfk-1 and lacking the usual low level of Pfk-2 (line 6) also grows on glucose. For the four strains with other than marginal phosphofructokinase level, growth rates and yields were similar. This result is confirmed in Table 2 which gives values found with independent isolates.

A similar pattern was found for aerobic growth on glucose-6-P (Table 1, column II): the four strains that grew on glucose also grew on glucose-6-P,

Table 2. Growth on glucose (0.4 mg/ml) of independent transductants (a,b, etc.) of 4 genotypes. Inocula were 1:100 from minimal medium with lactate (0.5 mg/ml). dt and Y as in Table 1.

Strain	dt	Y
DF531 (*pfkA*$^+$ *pfkB*$^+$)-a	82	4.0
" " -b	82	4.0
" " -c	82	4.0
DF533 (*pfkA1* *pfkB1*)-a	90	3.1
" " -b	100	4.1
" " -c	94	3.8
DF535 (*pfkA*$^+$ *pfkB1*)-a	82	4.4
" " -b	82	4.3
" " -c	82	4.2
" " -d	82	4.2
DF536 (*pfkA*$^+$ *pfkB1* *pfkB2*)-a	90	5.4
" " -b	76	4.6

with similar rates and yields. (The faster growth of the *pfkA* mutant on glucose-6-P than on glucose has been discussed (16).) Columns III and IV show that the strains that grew aerobically also grew anaerobically, on glucose and glucose-6-P. With the possible exception of the *pfkA1* *pfkB1* strain, the anaerobic growth rates and yields did not differ greatly among the strains.

Table 1 also shows that the six strains differ little in their growth on glycerol, aerobically (column V) or anaerobically (with added fumarate, column VI).

We conclude from these results that the allostery of phosphofructokinase is unlikely to be a primary factor governing the glycolytic pathway during growth of *E. coli*. Further comparative studies of these strains may be a useful way to identify those conditions where the allostery has a clear function in this prokaryote. It is also possible that major differences between the strains even in the present growth conditions will be revealed

by different measures (such as the amount of futile cycling, now under study).

Two other points merit consideration with respect to the allostery of phosphofructokinase, and its function. (i) The in vitro characteristics of the pure enzymes might accurately reflect their properties in the cell but the levels of substrates and effectors could be such that Pfk-1, in the given growth conditions, is in the allosterically active form and not very sensitive to changes in effector concentration. Lowry et al. (17) presented data to this point using parameters from ref. 3 together with in vivo metabolite levels (but see ref. (18)). J. Babul of this laboratory has obtained rate data on the pure enzyme using metabolite levels assessed in growth conditions and further such studies may be illuminating.

(ii) On the other hand, the known in vitro characteristics of the two enzymes may be misleading, either because there are unknown effectors or because their form is different in vivo. This possibility was considered in an interesting way by Reeves and Sols (18) who used toluenized wild type cells to assay phosphofructokinase "in situ". They found the kinetic characteristics to be generally similar to those known for pure Pfk-1, but with some differences, such as a Hill coefficient for fructose-6-P of 2 instead of 4. Mowbray and Moses have presented some evidence for a glycolytic complex in E. coli (19). Karadsheh and Uyeda have found that the membrane-bound erythrocyte phosphofructokinase, in contrast to the free enzyme, gives a non-sigmoidal saturation curve with fructose-6-P and is not inhibited by ATP (20).

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