Evidence for two distinct pyruvate kinase genes in *Escherichia coli* K-12

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A strain of *Escherichia coli* K-12 defective in pyruvate kinase F has been produced. The existence of this mutant, in conjunction with earlier results, strongly suggests that the two pyruvate kinases in this bacterium are distinct forms and not interconvertible. Either form of pyruvate kinase appeared to be equally effective in the glycolytic conversion of phosphoenolpyruvate to pyruvate. Genes specifying pyruvate kinase A and pyruvate kinase F were present on the small F-prime F506 and the locus for pyruvate kinase F was found to be at minute 36.5 on the *E. coli* genetic map.

Escherichia coli *K-12* *Genetic mapping* *Pyruvate kinase A* *Pyruvate kinase F*

1. INTRODUCTION

Multiple forms of the glycolytic enzyme pyruvate kinase (EC 2.7.1.40) have been observed in a wide range of cells and it seems likely that they play distinct physiological roles in metabolism [1]. In tissues such as liver and kidney and in many bacterial cells, where the processes of glycolysis and gluconeogenesis occur in the same cell, it is important to regulate these mutually opposing sequences and it seems that regulation of pyruvate kinase activity is significant in this respect. Two pyruvate kinases found in mammalian liver have been well-characterised and they are interconvertible forms of the same protein [2]. However, two enzymes from *Escherichia coli* K-12 have been purified and peptide mapping showed significant differences between them, suggesting that they were unlikely to be different forms of the same protein [3].

In [4] we described various properties of *E. coli* K-12 mutants defective in pyruvate kinase activity. However, the information then obtained was insufficient to disprove the existence of interconvertible forms of pyruvate kinase in this organism. Only two of the three possible pyruvate kinase mutants that should exist, if the two enzymes are quite separate, were then available. Mutants defective in both pyruvate kinase A and pyruvate kinase F (previously referred to as pyruvate kinase B [4]) or mutants defective in pyruvate kinase A only were obtained but no mutant lacking only pyruvate kinase F was isolated. Thus it was unlikely that pyruvate kinase A was modified to produce pyruvate kinase F since absence of A would be expected to lead to absence of F in such a situation. However, it was still possible that pyruvate kinase F could be modified to produce pyruvate kinase A since no mutant lacking F but still displaying A was obtained. The absence of pyruvate kinase A could then result from the inactivation of a system forming A from F. If this hypothesis is correct a mutant lacking pyruvate kinase F only (the third possible pyruvate kinase mutant) ought not to exist. The isolation of such a mutant would, on the other hand, support the view that pyruvate kinases A and F are quite separate proteins.

Here, we report the isolation of such mutants and show that a genetic locus specifying pyruvate kinase F is quite readily co-transducible with a gene for an analogous enzyme, phosphoenolpyru-

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vate synthase (EC 2.7.9.2) but unlinked by transduction to a gene specifying pyruvate kinase A.

2. MATERIALS AND METHODS

The *E. coli* K-12 strains used were AG 219 (F^-, *pykA*, *pykF*≡*pykB*) [4], NP37 (*pheS5*) [5] and the prototrophic strain K10 [6]. The *E. coli* K-12 F′ kit including F506 [7] was obtained from the *E. coli* Genetic Stock Center (Yale University). Bacteria were grown and assayed for pyruvate kinase as in [4]. However, when a large number of transductants were to be assayed it was necessary to use a modified procedure. For this bacteria were grown aerobically in 2 ml portions of glucose minimal medium in 125 mm × 16 mm test-tubes until they reached the late exponential phase of growth ($A_{680} \approx 0.8$). The 2 ml culture was then treated with toluene to permeabilise the cells (as in [8]) to permit the in situ assay of pyruvate kinase. The pyruvate kinase A activity was measured initially then the total pyruvate kinase activity (A + F) was measured after the further addition of fructose 1,6-bis phosphate to 1 mM [9]. Known mutants lacking pyruvate kinase A or pyruvate kinase F were included to check the reliability of the procedure.

Mutagenesis to yield phosphoenolpyruvate synthase mutants was as in [10] and transduction using phage P1 *vir* was by standard procedures [11]. Biochemicals were purchased from Boehringer Ltd. (London) and other reagents were of the highest purity commercially available.

3. RESULTS AND DISCUSSION

In a preliminary experiment to map the pyruvate kinase genes mutant AG 219 was mated with strains carrying different F-primes and the ability to grow on D,L-glycerate was selected. F 500 and F 506 but not F 123 or F 150 [12] were able to complement the pyruvate kinase mutations and when a number of independent F-ductants were grown and tested for pyruvate kinase activity some were found to have regained both pyruvate kinases whereas others had regained only one, either pyruvate kinase A or pyruvate kinase F. This suggested that either form of the enzyme was effective for the glycolytic reaction converting phosphoenolpyruvate to pyruvate. The isolation, for the first time, of strains defective only in pyruvate kinase F supported the view that the two pyruvate kinases were the products of distinct genes rather than interconvertible forms of one protein. When the pyruvate kinase F mutants were tested for growth on the various carbon sources used in [4] there was no difference in growth between pyruvate kinase F mutants and pyruvate kinase A mutants. This somewhat unexpected finding meant that we were unable to distinguish between pyruvate kinase A mutants and pyruvate kinase F mutants by simple plate growth tests.

Since F 506 was a short F-prime that was known to carry the *pps* gene it was thought that the pyruvate kinase genes might be close to *pps*. To test this possibility strain AG 219 was subjected to ethylmethane sulphonate mutagenesis and lactate-negative mutants were isolated. One such mutant, strain AG 239, was shown to lack phosphoenolpyruvate synthase activity. This strain was then used to study the co-inheritance of pyruvate kinase A and pyruvate kinase F. For this, strain AG 239 was F-duced with F 506 and lactate-positive colonies were selected. When 59 independent isolates were tested on lactose plates to detect the presence of pyruvate kinase all grew readily. Assay of pyruvate kinase activity showed that once again either of the enzymes singly, or both enzymes together, had been regained. Of the four different classes of recombinant expected (*pykA*, *pykF*; $pykA^+$ $pykF^+$; *pykA*, $pykF^+$; $pykA^+$, *pykF*) the first class was absent, suggesting that both pyruvate kinase genes were reasonably close to *pps* (table 1).

Strain AG 239 was used in phage P1 mediated transduction experiments to determine the extent of co-transduction between *pps* and *pykA* and *pykF*. Phage P1 grown on wild-type cells was used to select pps^+ transductants and these were subsequently tested for growth on lactose. About 25%

Table 1

F-duction of AG 239 (*pps*, *pykA*, *pykF*) by F 506 (pps^+, $pykA^+$, $pykF^+$)

Number of Pps^+ F-ductants that are:			
$pykA^+pykF^+$	$pykA^+pykF$	*pykA* $pykF^+$	*pykA* *pykF*
45	6	8	0

Table 2

Transduction of NP 37 (*pheS*) with phage grown on AG 239 (*pps*, *pykA*, *pykF*)

Number of *phe* S^+ transductants that are:			
pykF$^+$ *pps*$^+$	*pykF pps*$^+$	*pykF pps*	*pykF*$^+$ *pps*
40	1	19	81

(24/98) of the *pps*$^+$ transductants grew on lactose and pyruvate kinase assays showed that all had regained pyruvate kinase F only. The *pykF* gene was located more precisely by a 3-factor cross using phage grown on strain AG 239 with the temperature-sensitive mutant NP 37 (*pheS*) as recipient. Since *pykAF* double mutants grow normally on glucose [4] it was possible to obtain *pheS*$^+$ transductants by selection for growth on glucose at 42°C. Such transductants were then tested for their ability to grow on lactate and also assayed for pyruvate kinase activity. Table 2 shows that 100/141 transductants became lactate-negative and 20/141 became *pykF*-negative. Of the four possible classes of transductant expected from this three-factor cross *pykF*, *pps*$^+$ were much less frequent than the other three (table 2). On the assumption that the rare class was formed as a result of four cross-overs the order of the genes would be *pykF–pps–pheS*.

By use of an equation [13] to correlate genetic map distance with percentage co-transduction it seems that *pykF* is 0.7 minute from *pps* at minute 36.5 or minute 37.9 and 1.0 minute from *pheS* at minute 36.5 or minute 38.5. The gene order suggests that *pykF* is at minute 36.5 on the *E. coli* genetic map [14]. Since no co-transduction between *pykA* and *pheS* or *pps* was observed the precise location of *pykA* is still unknown.

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