CONTROL *IN SITU* OF THE PYRUVATE KINASE ACTIVITY OF *ESCHERICHIA COLI*

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1. Introduction

Extracts of *Escherichia coli* contain two forms of pyruvate kinase (EC 2.7.1.40) which can be separated from each other by chromatography [1]. The enzymic activity of one form, eluted by 0.12 M KCl from columns of DEAE-cellulose, is analogous to that of the pyruvate kinase of yeast [2]: it is greatly stimulated by fructose 1,6-diphosphate when one of the substrates, phosphoenolpyruvate (PEP) is present at low concentrations. The second type of pyruvate kinase, eluted from DEAE-cellulose at 0.18 M KCl, was found to be rather similar to the pyruvate kinase of muscle, which is not activated by fructose 1,6-diphosphate. However, unlike that of the muscle enzyme, the activity of this form of pyruvate kinase is stimulated by AMP [3, 4].

It is difficult to assess the physiological significance to *E. coli* cells of events observed in spectrophotometer cells, especially since the concentrations of regulatory enzymes in these two types of cell may differ by as much as a million-fold [5]. A means of studying such enzymes in *E. coli*, 'permeabilized' by treatment with toluene followed by freezing and thawing, has recently been developed by Reeves and Sols [6]; this treatment permits enzymes to be studied "*in situ*", at the concentrations normally present in the intact organism. Regulatory properties revealed under such conditions should, therefore, be free from many of the objections that can be levelled at studies of dilute enzymes in aqueous solution; moreover, they permit of comparison of the two types of study.

It is the main purpose of this paper to describe the parameters that affect the enzymic conversion of PEP and ADP to pyruvate and ATP *in situ* by 'permeabilized' *E. coli*. As reported from studies with isolated enzymes [1], the results show that the total pyruvate kinase activity of *E. coli* grown on glycolytic substrates is considerably greater than that of cultures grown on gluconeogenic substrates; that the form of pyruvate kinase activated by fructose 1,6-diphosphate (henceforth designated Pyk-F) plays a predominant role under the former growth conditions whereas the AMP-stimulated form (Pyk-A) is of paramount importance under the latter; and that, in the range of PEP concentrations likely to occur in *E. coli* [7], pyruvate kinase activity is significantly manifested only if either AMP or fructose 1,6-diphosphate is also present.

2. Materials and methods

Cultures of the *E. coli* K12 strain K10 (Hfr-C) were grown at 37° either aerobically with shaking on media containing salts [8] and a carbon source (at 25 mM), or, in standing culture, anaerobically, in similar medium containing 20 mM glucose. The cells were harvested, washed, and 'permeabilized' as prescribed by Reeves and Sols [6]. The pyruvate kinase activities of the cell suspensions thus obtained were measured at 30° in cuvettes containing, in 1 ml: 20 μmoles of imidazole buffer, pH 7.3; 5 μmoles of $MgCl_2$; 50 μmoles of KCl; 5 μg of lactate dehydrogenase (EC1.1.1.37); 0.1 μmole of NADH; 0.1 – 1.0 μmole of PEP; 3 μmoles of ADP; and cells (0.05–0.08 mg dry mass). As appropriate, 1 μmole of fructose 1,6-diphosphate, or 3 μmoles of AMP, were also added; the reaction was started by the addition of ADP and was measured as the rate of decrease in

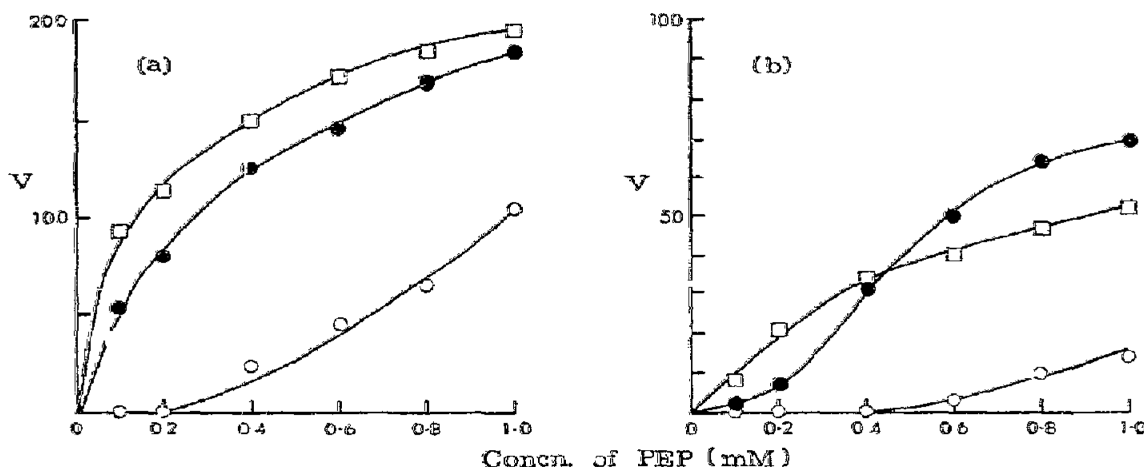

Fig. 1. Effect of PEP concentration on the pyruvate kinase activity of suspensions of 'permeabilized' *E. coli*, that had been grown (a) anaerobically, on glucose, and (b) aerobically, on acetate, as sole carbon sources. For each assay, 0.05 mg dry mass of cells were used in (a), 0.08 mg in (b). Pyruvate kinase activities were measured in the absence of effectors (o—o—o), and in the presence of 3 mM AMP (•—•—•) or 1 mM fructose 1,6-diphosphate (□—□—□); the rates (V) are expressed as nmoles of NADH oxidized/mg dry mass/min.

extinction at 340 nm concomitant with the reduction of pyruvate (formed from PEP) to lactate. All measurements were corrected for the (low) NADH-oxidase activities of the cell suspensions.

3. Results and discussion

The pyruvate kinase activities of 'permeabilized' *E. coli* suspensions remained wholly associated with the cells, and none was detected in the supernatant solutions obtained after centrifuging such suspensions (at 1 mg dry mass/ml). Exposure of the suspensions to ultrasound released the enzymes into solution: the quantities thus released ("*in vitro*") were roughly those assayed *in situ*. Apart from the low rates of NADH oxidation associated with traces of NADH-oxidase activity, significant decreases in extinction at 340 nm were observed only if the required reactants — phosphoenolpyruvate, cells, ADP, and lactate dehydrogenase — were also present. Furthermore, the rates of pyruvate kinase activity thus measured were stoicheiometric with time, and with the quantities of cell suspensions added over the range 0.01–0.08 mg dry mass/ml.

As shown in fig. 1a, cells grown under extreme glycolytic conditions — anaerobically, on glucose as sole carbon source — contained high total activities of pyruvate kinase. However, in the 'physiological' range [7] of substrate concentrations here used for study, this enzymic activity was not readily detectable at concentrations of PEP at or below 0.2 mM unless either AMP or fructose 1,6-diphosphate was also present. Increasing the quantities of ADP added (to 10 mM) was wholly ineffective in eliciting pyruvate kinase activity in the absence of AMP, and the stimulation produced by AMP was identical when 10 mM ADP was present to that normally observed with 3 mM ADP. The enzymic activity elicited by the addition of 1 mM fructose 1,6-diphosphate (Pyk-F) was consistently higher than that observed after addition of 3 mM AMP (Pyk-A); Lineweaver-Burk plots of both these activities were linear, with K_m values for PEP of approx. 0.2 mM (Pyk-F) and 0.5 mM (Pyk-A), respectively. These K_m values are very close to those measured *in vitro* with the purified enzymes [4].

In contrast, the total pyruvate kinase activity *in situ* of cultures grown on gluconeogenic substrates was much less than that of glucose-grown cells. This is illustrated by the results obtained with cells grown on acetate. The total pyruvate kinase activity measured was only about one-third that of glucose-grown cells (fig. 1b), which confirmed the reported inducibility of the pyruvate kinase(s) of *E. coli* [1]. Again,

in the absence of either AMP or fructose 1,6-diphosphate, little or no enzymic activity was detected at PEP concentrations at or below 0.4 mM; in contrast to the results obtained with glucose-grown cells, the activity of Pyk-F in acetate-grown cells was less than that of Pyk-A at concentrations above 0.4 mM. This difference in the relative activities of Pyk-F and Pyk-A makes it unlikely that, as postulated by Maeba and Sanwal [3], the stimulations by AMP and fructose 1,6-diphosphate are exerted on the same form of pyruvate kinase; a further difference is revealed by the markedly sigmoid plot of Pyk-A activity observed *in situ* in acetate-grown cells (fig. 1b) and in ultra-sonic extracts obtained from them. However, a Lineweaver–Burk plot of Pyk-F activity remained linear, with K_m for phosphoenolpyruvate of approx. 0.2 mM.

Measurements with glucose-grown cells (fig. 1a) and with purified enzymes [4] show that the K_m for phosphoenolpyruvate of Pyk-F is less than that of Pyk-A. The significance *in situ* of this difference is illustrated in fig. 1b: at phosphoenolpyruvate concentrations at and below 0.4 mM, the plot of Pyk-A activity falls below that of Pyk-F, which shows that the affinity of this type of pyruvate kinase for this substrate observed *in situ* reflects that observed *in vitro*. It thus appears that, at least for the pyruvate kinases of *E. coli*, the 'coarse' and 'fine' controls postulated from measurements with dilute, isolated enzymes [1, 4] operate also under the conditions obtaining in the undisrupted but 'permeabilized' cells.

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