DIMERIC AND TETRAMERIC PHOSPHOFRUCTOKINASE AND THE PASTEUR EFFECT IN *ESCHERICHIA COLI* K-12

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1. Introduction

The Pasteur effect, that is the effect of oxygen on glucose metabolism, in muscle tissue [1] and bacteria [2–4] was always thought to be due to a rise in the ATP concentration in the cell which, in turn, inhibits phosphofructokinase activity. Continuous culture experiments, however, have shown that oxygenation of the facultative anaerobe *E. coli* K-12 lead to a reduction in the rate of phosphofructokinase synthesis [5]. Enzymatic investigations revealed further that the kinetics of the enzyme phosphofructokinase from strictly aerobic cultures (6.9 ppm oxygen in solution) was ATP-insensitive, whereas the same enzyme from anaerobic cultures was ATP-sensitive [6,7]. The purification and kinetic characterization of these two forms revealed that the enzyme phosphofructokinase exists as a dimer (mol. wt 150 000) in aerobic cultures and as a tetramer (mol. wt 350 000) under anaerobic conditions [8]. This communication describes the role played by these two forms of phosphofructokinase in the effect of oxygen on the glucose metabolism of *E. coli* K-12 in relation to the mechanism of the Pasteur effect.

2. Materials and methods

All reagents used were of A. R. grade. Ammonium sulfate was special enzyme grade from Schwarz-Mann (Orangeburg, New York), while reagents for preparing acrylamide gels, phenazine methosulfate, nitro-blue tetrazolium and all nucleotides were purchased from Serva Feinbiochemica. Fructose 6-phosphate, glycine and protamine sulfate were obtained from Sigma Chem. Co. (U.S.A.), the auxiliary enzymes and protein standards for molecular weight determinations from Boehringer (Mannheim, Germany).

Culture conditions of *E. coli* K-12, as well as the arrangements for the continuous culture, were identical to those conditions described by Reichelt and Doelle [5].

Cell free extract preparations and protamine sulfate precipitation were carried out as described by Doelle [8].

Ammonium sulfate treatment. To the supernatant from the protamine sulfate treatment was added slowly with gentle stirring solid ammonium sulfate (224 g/l) to give a 40% saturation. The preparation was allowed to stand for 15 min before the precipitate was removed by centrifugation at 37 000 *g* for 20 min and discarded. To this supernatant further ammonium sulfate was added to give a 80% saturation. The precipitate was collected by centrifugation at 56 000 g for 20 min and dissolved in buffer containing 0.1 M Tris–HCl (pH 7.0), 2 mM 2-mercaptoethanol and 0.45 mM fructose 6-phosphate.

Enzyme assays, protein estimations, polyacrylamide gel electrophoresis and the determination of molecular weights were carried out as described previously [8].

3. Results and discussion

The results of this investigation taken from 13 different steady states between the input partial pressures of oxygen (pO_2) of 0 to 200 mm Hg are given in figs. 1 and 2. They demonstrate that the input partial pressure of oxygen is responsible for the change

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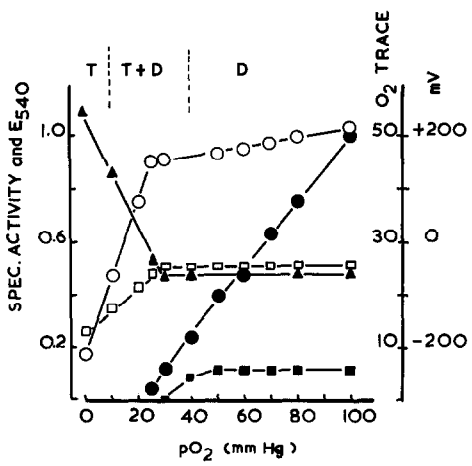

Fig. 1. The effect of input partial pressure of oxygen on the specific phosphofructokinase activity (▲–▲–▲), cell density (□–□–□), α-ketoglutarate dehydrogenase (■–■–■), dissolved oxygen tension (●–●–●) and mill-volt (○–○–○) in a glucose-limited chemostate culture of *E. coli* K-12 with a dilution rate of 0.2 hr^{-1} and pH 7.0 at 37°C. T: Tetramer; D: Dimer.

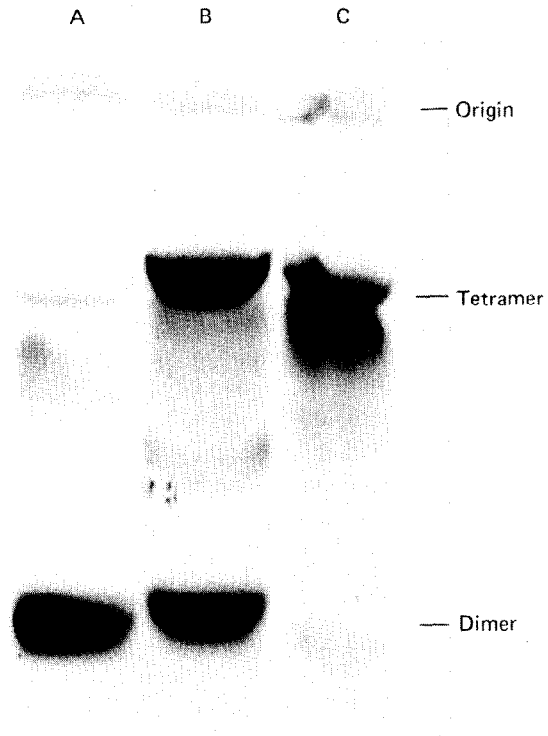

Fig. 2. Polyacrylamide gel (10%) electrophoresis of partially purified phosphofructokinase obtained from cultures of *E. coli* K-12 grown at various steady states in a glucose-limited chemostat with a dilution rate of 0.2 hr^{-1} and pH 7.0 at 37°C. A: pO_2 of 0 mm Hg; B: pO_2 of 20 mm Hg; C: pO_2 of 200 mm Hg.

of the phosphofructokinase protein from a dimeric to the tetrameric form. This change occurs via an intermediate stage, in which both forms are equally present. It has been reported [6–8] that the aerobic or dimeric form is ATP-insensitive, whereas the tetrameric or anaerobic form shows the well-known ATP-sensitivity.

In the intermediate stage with both forms present, allostery dominates. The tetameric form (fig. 2c) was found between pO_2 0 and 5 mm Hg, the intermediate stage with both forms (fig. 2b) at 10, 20, 25 and 30 mm Hg, whereas the dimeric form (fig. 2a) prevailed at 40 mm Hg and higher.

These changes of the phosphofructokinase protein have, however, to be seen in the light of other parameters given in fig. 1. All of these results confirm our earlier reports [6]. Reducing the oxygen input partial pressure to 40 mm Hg, the first indication of oxygen restriction becomes visible by the reduction of α-ketoglutarate dehydrogenase activity, which becomes zero at 30 mm Hg. With the cessation of the synthesis of this enzyme, the TCA cycle becomes a branched pathway and the cell is not able to produce the amount of ATP required for aerobic respiration, thus fermentation starts, which is indicated by the start of acid production [5,6]. It is this steady state of pO_2 (30 mm Hg) that both forms of phosphofructokinase protein appear for the first time. This steady state is also important in regard to the dissolved oxygen tension, since the affinity of the cell for oxygen changes and can not be treated as a simple Michaelis-Menten relationship [9–11]. A sharp drop on the millivolt scale and the start of increased phosphofructokinase synthesis [5] also occur, together with a decline in cell population. It is not until the pO_2 drops below 10 mm Hg that the dimeric form is completely replaced by the tetrameric form. In the reverse cycle, the dimeric form first appears at a pO_2 of 10 mm Hg.

These investigations revealed that the mechanism of the Pasteur effect can not be related to the allostery of the enzyme phosphofructokinase. The reduction of ATP production due to the cessation of the TCA cycle possibly induces the synthesis of the tetrameric form, which regulates the energy metabolism of the

anaerobic cell. This shift of energy regulation from the enzymes of the TCA cycle during aerobiosis to phosphofructokinase is necessary because of the cessation of aerobic respiration and the TCA cycle under anaerobic conditions. Although the possibility of an association or dissociation of the tetramer to and from an inactive form can not be ruled out, it is suggested that the cell induces a separate dimeric form of phosphofructokinase depending upon the prevailing oxygen conditions.

It is therefore proposed that this shift in energy regulation caused by the induction of synthesis of one and the repression of the second form of phosphofructokinase protein represents the mechanism of the Pasteur effect. Work is in progress to establish the inducer and repressor of this mechanism.

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