Short Communications

SC 2240

Fructose 1,6-diphosphate breakdown in *Escherichia coli*

In an earlier report from this laboratory[1], it was shown that the reduction of Fru-6-*P* to mannitol-6-*P* plays an important part in the reoxidation of NADH to NAD^+ in the anaerobic glycolysis of *Escherichia coli*. In these experiments whole cells and centrifuged extracts were incubated with $^{32}P_i$. It was noted that under anaerobic conditions Fru-1,6-P_2 had a much higher isotope concentration than a mixture of hexose 6-phosphates. This was not the case under aerobic conditions.

Several reports in the literature have stressed the importance of Fru-6-*P* phosphorylation in the regulation of the rate of glycolysis[2]. Other factors influencing the concentration of Fru-1,6-P_2 are probably also important, and in this connection the enzymes catalyzing the hydrolysis of the sugar phosphates are of interest[3]. In view of our previous findings[1] we have attempted to evaluate the extent to which hydrolysis of Fru-1,6-P_2 in the 1 position may be responsible for the rapid turnover of this substance in our experiments. Whole cells and cell-free extracts of *E. coli* were used.

In Table I are given the results of some experiments with a cell-free extract. The chromatographic procedure has been described previously[1]. Pyruvic acid was determined according to KOEPSELL AND SHARPE[4].

TABLE I

AMOUNTS OF GLYCOLYTIC INTERMEDIATES FOUND IN A CELL-FREE EXTRACT OF *E. coli*

Each flask contained 95 ml of cell-free extract corresponding to 12 g of cells, 3 mmoles Tris buffer (pH 7.7), and 1 g of glucose. The anaerobic flasks were incubated under reduced pressure, and the aerobic flask was shaken with oxygen for 15 min at 37°. Trichloroacetic acid was added at the end of the incubation, and the P compounds were precipitated with barium acetate and alcohol before separation by chromatography[1].

Conditions	Added (μmoles)		Found (μmoles)			
	ATP	P_i	Hexose 6-P	Mannitol 1-P	Fru-1,6-P_2	Pyruvate
Aerobic	0	25	36.5	8.8	4.5	48
Anaerobic	0	25	25.5	20.8	—	19
Anaerobic	50	25	36.5	99.8	4.9	173
Anaerobic	50	525	36.5	100.0	4.0	221
Unincubated						6

In Expt. 2, with anaerobic incubation and no addition of ATP, the concentration of Fru-1,6-P_2 was so low that it could not be isolated. Only small amounts of pyruvic acid and mannitol-1-*P* were present, indicating a low rate of glycolysis[1]. The amount of hexose 6-phosphates isolated under these conditions, however, was only slightly lower than that found either aerobically, or under anaerobic conditions with ATP.

Judged from the amounts of pyruvic acid and mannitol 1-*P* found in Expts. 3 and 4, the glycolytic processes must have been greatly enhanced by the addition of ATP, while P_i had little effect. It should be noted that the extract rapidly hydrolyzed the added ATP, so that most of the adenine nucleotide recovered was in the form of ADP and AMP. As expected, only a small amount of mannitol-1-*P* was found in the aerobic experiment[1]. The low concentration of pyruvic acid found in this experiment may probably be explained by a removal of pyruvate by oxidative breakdown, but may also partly be due to a lower rate of glycolysis under aerobic conditions.

The activities of the phosphatases attacking Fru-1,6-P_2 and Glu-6-*P* were measured in the extract which was used in the experiments of Table I, at the same pH and with the same buffer (Tris). It was found that 1 ml of extract was able to hydrolyze 5.9 μmoles of Fru-1,6-P_2, and 1.0 μmole of Glu-6-*P* in 15 min. In the experiments described in Table I, 78 ml of extract were used in each case, and the incubation lasted for 15 min. Therefore, a maximum of 460 μmoles of Fru-1,6-P_2, and 80 μmoles of Glu-6-*P* could have been hydrolyzed during the incubation. In Expts. 3 and 4, a minimum of 100 μmoles of Fru-1,6-P_2 were broken down to form 200 μmoles of pyruvic acid. At least 200 μmoles of hexose 6-phosphate were needed to form 100 μmoles of Fru-1,6-P_2 and 100 μmoles of mannitol-1-*P*. These data show that the extract had the capacity to break down Fru-1,6-P_2 more than 4 times faster by hydrolysis than by the glycolytic pathway. This was not the case with hexose 6-phosphate, of which glycolytic processes used at least 200 μmoles, while hydrolysis removed not more than 100 μmoles.

In Fig. 1 are presented the data from an experiment with intact cells of *E. coli*. Separate data for the two phosphate groups in Fru-1,6-P_2 were obtained by treating the purified compound with 1 N HCl at 100° for 15 min, and then chromatographically separating the products: Fru-6-*P*, P_i, and some unchanged Fru-1,6-P_2. The two phosphate groups of Fru-1,6-P_2 had about the same specific activity. This shows that

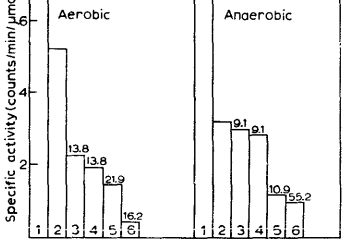

Fig. 1. Specific activities of P compounds from *E. coli*. Column 1, P_i; Column 2, ATP; Column 3, Fru-1,6-P_2, position 1; Column 4, Fru-1,6-P_2, position 6; Column 5, hexose 6-phosphate; Column 6, mannitol-1-*P*. The numbers over the columns give the amounts isolated of each compound in μmoles. Each flask contained 25 g of cell paste, 400 mg of glucose, and water to 95 ml. $^{32}P_i$ was added at zero time. Experimental procedure as in Table I.

the aldolase (EC 4.1.2.7)–triose-*P* isomerase (EC 5.3.1.1) catalyzed reactions operate fast enough to randomize the P groups. Combined with the fact that Fru-1,6-P_2 may be rapidly hydrolyzed in the 1 position, and again rephosphorylated, this explains why Fru-1,6-P_2 may have an average specific activity approaching that of the terminal P group of ATP.

The specific activity of the two P groups of Fru-1,6-P_2 were higher under anaerobic than under aerobic conditions, both absolutely, and relative to the parent compounds, ATP and hexose 6-phosphate. A more rapid synthesis and breakdown of Fru-1,6-P_2 must therefore have taken place under anaerobic conditions. This may be due to a more rapid glycolysis in the absence of oxygen, but this would require a more rapid turnover of hexose 6-phosphate, both for the formation of Fru-1,6-P_2, and for the increased mannitol-1-*P* formation (Fig. 1). It therefore seems as if additional factors are needed to explain the increased turnover of Fru-1,6-P_2 under anaerobic conditions, and hydrolysis in the 1 position may offer a reasonable explanation for the observations. Increased Fru-1,6-P_2 concentration under anaerobic conditions might lead to increased rate of hydrolysis, but no systematic increase in Fru-1,6-P_2 concentration has been observed in our experiments so far (Table II).

TABLE II

AMOUNTS OF Fru-1,6-P_2 ISOLATED FROM SUSPENSIONS OF *E. coli* CELLS, IN PARALLEL EXPERIMENTS UNDER AEROBIC AND ANAEROBIC CONDITIONS

The data are taken from ref. 1 and Fig. 1.

Expt.	*Fru-1,6 P_2 (μmoles)*	
	Aerobic	*Anaerobic*
1	2.15	2.15
2	2.73	4.88
3	13.8	9.1

It therefore seems possible that D-Fru-1,6-P_2-1-phosphohydrolase (EC 3.1.3.11) is influenced, either by changes in oxygen tension, or by the events accompanying such a change, such as variations in the concentrations of the adenine nucleotides and of P_i. These problems are at present under investigation in our laboratory.

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