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STRINGENT CONTROL OF GLYCOLYSIS IN ESCHERICHIA COLI

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SUMMARY: When Escherichia coli CP78(rel$^+$) growing on glucose was starved for isoleucine by the addition of valine, the intracellular levels of fructose 6-phosphate, fructose 1,6-bisphosphate and dihydroxyacetone phosphate were abruptly decreased to one-half, but those of glucose 6-phosphate and ATP remained constant. In contrast, this was not the case with CP79(rel$^-$). Chloramphenicol released the response observed in CP78. These results suggest that the glycolytic activity is also under the stringent control. Since only glucosephosphate isomerase[EC 5.3.1.9] was significantly inhibited by guanosine 5'-diphosphate 3'-diphosphate among several glycolytic enzymes tested, the enzyme might be responsible for the decrease observed in CP78.

The term "stringent control" originally described the phenomenon that the rate of RNA synthesis is reduced in bacterial cells when they are starved for any required amino acid, and the phenomenon was pointed out to be governed by rel gene (1). In recent years, not only RNA synthesis but also various other cellular processes have been shown to be under the stringent control, such as syntheses of lipids, nucleotides, polyamines (2,3) and peptidoglycans (4), and uptakes of purines, pyrimidines and α-methyl-D-glucoside (2,3). Now it is established that rel gene is involved in the formation of ppGpp and guanosine 5'-triphosphate 3'-diphosphate, which accumulate abruptly in the cells when they are starved for the amino acid. The compounds are now being considered to mediate the stringent responses (2,4).

Recently, we demonstrated that ppGpp is one of the activators of phosphoenolpyruvate carboxylase [EC 4.1.1.31] of E. coli (9), which plays a key role in connecting the glycolytic pathway with the TCA cycle and is

Abbreviations: ppGpp, guanosine 5'-diphosphate 3'-diphosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; G6P, glucose 6-phosphate; DHAP, dihydroxyacetone phosphate; F6P, fructose 6-phosphate; CAP, chloramphenicol.

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controlled by various kinds of allosteric effectors (5-8). This finding led us to investigate the intracellular levels of the effectors and substrate of the enzyme and to examine whether the glycolysis is also under the stringent control. As a result of experiments, the levels of Fru-1,6-P_2, one of the activators of the enzyme, and some other glycolytic intermediates in E. coli were found to be decreased when the cells were starved for isoleucine, suggesting that the glycolysis is under the stringent control. The effects of ppGpp on the activities of some glycolytic enzymes were also examined to disclose the enzyme(s) responsible for the stringent response considered.

MATERIALS AND METHODS

E. coli, CP78 and CP79, were used which are an isogenic pair of rel^+ and rel^-, respectively. They require histidine, leucine, threonine, arginine and thiamine for growth and are sensitive to valine inhibition (10,11). The growth medium contained the following constituents per liter; 7g of K_2HPO_4, 3g of KH_2PO_4, 1.1g of NH_4Cl, 0.1g of $MgSO_4 \cdot 7H_2O$, 4g of D-glucose, 1 mg of thiamine hydrochloride and 20 mg each of the required amino acids.

The bacteria were grown at 30°C under forced aeration. Growth was monitored by measuring the absorbance of the culture at 660nm. The absorbance of 0.23 (1-cm light path) corresponded to 0.1 mg of the cells in dry weight per ml. The samples for analysis of metabolites were prepared as described by Lowry et al. (12) with some modifications. The cells at logarithmic growth phase (A_{660} =0.2) were collected on Millipore filter whithin one minute and were frozen with Freon 12. The metabolites were extracted from cells with 0.4M $HClO_4$ and the extract was neutralized with 0.2M K_2CO_3(13). The precipitated $KClO_4$ was removed by centrifugation and the supernatant fluid was stored at -80°C for the analysis.

ATP, G6P, Fru-1,6-P_2, DHAP and non-adenine nucleoside triphosphates were determined by the fluorometric method of Lowry et al.(12). F6P, 6-phosphogluconate and acetyl-CoA were also determined fluorometrically according to the methods of Lang et al. (14), Haid (15) and of Williamson et al. (16) with some modifications, respectively.

All the authentic samples of metabolites and the enzymes used for fluorometric analyses were purchased from Sigma and Boehringer Mannheim, respectively. ppGpp was purchased from Kyowa Hakko Kogyo, Tokyo. All other chemicals were of reagent grade.

RESULTS AND DISCUSSION

In the present investigation, E. coli, CP78 and CP79, were starved for isoleucine by the addition of valine, which acts as a feedback inhibitor of isoleucine biosynthesis (10,11). This method has an advantage that the

starvation can be rapidly imposed on cells without unnecessary perturbation of cellular metabolism. Figure 1 shows time course of the changes in intracellular levels of several metabolites after the addition of valine to the cultures of CP78 and CP79 growing on glucose as a sole carbon source. The levels of F6P, Fru-1,6-P_2 and DHAP in CP78 decreased to one-half in a few minutes, whereas those of G6P and ATP remained virtually constant (Fig. 1A). The levels of acetyl-CoA, 6-phosphogluconate and non-adenine nucleoside triphosphates (1.0, 0.75 and 2.5 μmol per g of the cells in dry weight, respectively) also remained constant. With CP79, on the other hand, the levels of F6P, Fru-1,6-P_2 and DHAP did not decrease but rather showed a tendency to increase slightly (Fig. 1B). Also when the starvation was imposed by depriving all the required amino acids, an abrupt decrease in the levels of Fru-1,6-P_2 and DHAP was observed in CP78 but not in CP79 (data not shown). These results indicate that decreases of F6P, Fru-1,6-P_2 and DHAP in CP78 were due to the stringent response.

Since CAP is known to be able to release the stringent control (2,3), the influence of CAP on the valine effect in CP78 cells was examined. As can be seen in Fig. 2A, no decrease but rather a slight increase in the levels of F6P, Fru-1,6-P_2 and DHAP was observed when valine and CAP were added simultaneously. When CAP was added 10 min after the addition of valine, the reduced levels of Fru-1,6-P_2 and DHAP were raised to the levels that were attained in the experiment of simultaneous addition (Fig. 2B). Conversely, when valine was added after the addition of CAP, no decrease in the levels was observed (data not shown). These results again indicate that the observed effect of valine on the levels of glycolytic intermediates was due to the stringent response.

Dietzler et al. reported that the rate of glucose utilization was reduced upon nitrogen limitation in E. coli cells, and that the rate paralleled the cellular level of Fru-1,6-P_2 (17). In one of their experiments, the level of Fru-1,6-P_2 in threonine-limited cells of E. coli G34,

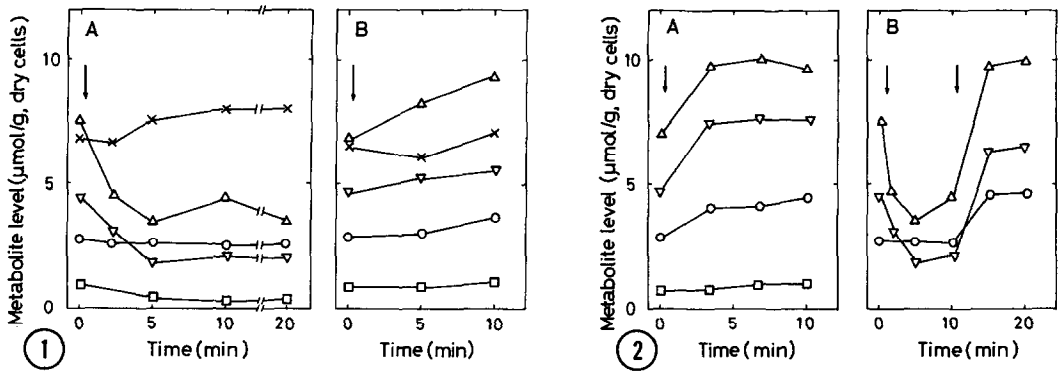

Fig. 1. Effect of valine addition on the intracellular levels of some metabolites. Valine (250 μg per ml, in final concentration) was added to the cultures of CP78 (A) and CP79 (B) in the logarithmic growth phase at the time shown by arrows. (—○—), G6P; (—□—), F6P; (—△—), Fru-1,6-P_2; (—▽—), DHAP; and (—×—), ATP.

Fig. 2. Influence of CAP on the valine effect in CP78. In A, valine and CAP were added to the culture simultaneously. In B, valine was added and, after 10 min, CAP was added as indicated by arrows. The final concentrations of valine and CAP were 250 and 50 μg per ml, respectively. (—○—), G6P; (—△—), Fru-1,6-P_2; and (—▽—), DHAP.

a "leaky" strain for threonine, was shown to be lower than that in the fully alimented cells. These results were in agreement with ours. However, they did not relate these results to the stringent control. We conducted a series of short term experiments in which the change in the levels of several metabolites including Fru-1,6-P_2 was followed for 20 min after the valine addition and disclosed that the abrupt decreases in F6P, Fru-1,6-P_2 and DHAP were due to the stringent response.

Sokowa et al. observed that the rate of ^{14}C-incorporation from [^{14}C]-glucose into various cellular constituents was reduced under the stringent control (18), and they indicated that the depression of glucose transport activity might be responsible for this phenomenon (19). In these experiments, however, the starvation was imposed by deprivation of all the required amino acids, while in our present experiments it was imposed by the valine addition. We examined whether the rate of α-methyl-D-glucoside transport was reduced by the valine addition. But no significant reduction of the transport rate

Table I. Effect of ppGpp on the activity of glucosephosphate isomerase. E. coli K12 cells grown on glucose were harvested by centrifugation and were suspended in 0.1M Tris-HCl buffer, pH 7.4. The cells were disrupted by sonication and were centrifuged at 20,000xg for 20 min to obtain a supernatant. Glucosephosphate isomerase was partially purified by ammonium sulfate fractionation (65-80%) and DEAE-Sephadex A-50 column chromatography (specific activity, 18 units/mg of protein). The enzyme activity was determined by the measurement of NADPH formation at 340 nm in a coupled reaction system with G6P dehydrogenase at 30°C. The reaction mixture contained the following constituents in μmol in a total volume of 1.0 ml: Tris-HCl buffer, pH 7.4, 100; $NADP^+$, 1.0; F6P, 0.5; 1.4 IU of G6P dehydrogenase; the partially purified enzyme (2.8 μg of protein); and ppGpp as indicated.

Addition (mM)	Activity(ΔA_{340}/min)	Inhibition(%)
None	0.18	0
ppGpp (1.0)	0.11	39
ppGpp (2.0)	0.07	61

occurred. This confirms Sokawa's unpublished observation that the extent of the reduction was not so significant when the valine addition method was used instead of the deprivation method. Further studies are necessary for elucidation of the stringent control of glucose transport activity under our experimental conditions. In any event, our observation that the levels of F6P, Fru-1,6-P_2 and DHAP were abruptly decreased upon amino acid starvation while the level of G6P remained constant suggests that the glycolysis is regulated by some mechanism after glucose is transported into the cells.

The rapidity of the response would imply the functioning of some post-translational regulatory mechanism of the enzyme(s) which is involved in the metabolism around F6P. This led us to examine the effect of ppGpp on the activities of some partially purified glycolytic enzymes. As seen in Table I, the activity of glucosephosphate isomerase[EC 5.3.1.9] which was measured in a direction of G6P formation from F6P was significantly inhibited by ppGpp at the concentrations found in the cells. The activity which was measured in the reverse direction was also inhibited to the same extent (data not shown). The inhibition was of non-competitive type with the substrate. The

activities of other enzymes such as phosphofructokinase[EC 2.7.1.11], aldolase[EC 4.1.2.13], and G6P dehydrogenase[EC 1.1.1.49] were scarcely affected by the addition of 1 mM ppGpp. If glucosephosphate isomerase is inhibited by ppGpp in vivo, the decreases of the glycolytic intermediates under the stringent control can be consistently understood. Further studies are now in progress to elucidate whether the other regulatory mechanism is also involved in this phenomenon.

Recently we reported that phosphoenolpyruvate carboxylase was activated by ppGpp in an allosteric manner (9). In consideration of this observation, together with the present one, it is quite possible that central pathways such as the TCA cycle and the glycolytic pathway are also regulated by the stringent control.

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