ACTIVATION OF *ESCHERICHIA COLI* PHOSPHOENOLPYRUVATE CARBOXYLASE BY GUANOSINE-5′-DIPHOSPHATE-3′-DIPHOSPHATE

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Received 21 March 1977

1. Introduction

Bacteria such as *Escherichia coli* respond to amino acid starvation by reducing the rate of syntheses of RNA, lipids, nucleotides and glycolytic esters [1,2]. This phenomenon is termed 'stringent response'. It has been pointed out that, concomitant with this cellular response, a sharp increase in the intracellular levels of guanosine-5′-diphosphate-3′-diphosphate (ppGpp) and guanosine-5′-triphosphate-3′-diphosphate (pppGpp) occurs [3]. These nucleotides are now being thought to mediate various stringent responses mentioned above. In fact, it has been reported that ppGpp directly inhibits several enzymes involved in lipid and nucleotide syntheses, such as acetyl CoA carboxylase [4], *sn*-glycerol-3-phosphate acyltransferase [5], and phosphoribosyltransferase [6]. However, no report seems to have appeared on the effects of ppGpp on the activities of enzymes involved in glycolysis or the tricarboxylic acid cycle.

Phosphoenolpyruvate carboxylase (EC 4.1.1.31) of *Escherichia coli* is an allosteric enzyme which plays an anaplerotic role by replenishment of oxaloacetate to the tricarboxylic acid cycle [7]. The enzyme is activated by acetyl CoA [8], fructose-1,6-bisphosphate [9] and fatty acids (or their CoA derivatives)[10] and is inhibited by L-aspartate (or L-malate) [11]. Furthermore, the enzyme is activated by GTP or CDP (unpublished work), similarly to the enzyme of *Salmonella typhimurium* [12]. This communication describes that ppGpp is the most potent activator of phosphoenolpyruvate carboxylase among the guanine nucleotides tested and discusses its possible physiological role.

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine *N*′-2-ethanesulfonic acid; PEP, phosphoenolpyruvate; FBP, fructose-1,6-biphosphate; CoASAc, acetyl CoA; ppGpp, guanosine-5′-diphosphate-3′-diphosphate; pppGpp guanosine-5′-triphosphate-3′-diphosphate.

2. Materials and methods

Partially purified PEP carboxylase (spec. act. 15 units/mg protein) of *E. coli* K12 (strain W3110) was used throughout this work. [13]. The enzyme activity was measured by the spectrophotometric method [13]. The standard reaction mixture contained the following constituents in total vol. 1.0 ml: 0.1 M HEPES–KOH buffer, pH 7.3, 0.2 mM potassium PEP, 10 mM $MgSO_4$, 10 mM $KHCO_3$, 0.1 mM NADH, 5 IU malate dehydrogenase (EC 1.1.1.31) and the enzyme (20 μg protein). Protein concentration was determined by the method of Lowry et al. [14]. GMP, GDP, GTP, and HEPES were purchased from Sigma Chemical Co. and ppGpp and pppGpp were from Kyowa Hakko Co. Ltd. All other reagents were prepared or purchased as described previously [13].

3. Results

Table 1 shows an effect of ppGpp on PEP carboxylase activity under various conditions. The enzyme was activated about 3-fold by ppGpp when assayed in the presence of 0.2–10 mM PEP. The extent of activation showed a tendency to increase with increasing concentrations of PEP. In the presence of 0.2 mM PEP which

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Table 1
Activation of PEP carboxylase by ppGpp under various conditions

Concentration of PEP (mM)	Addition (mM)	Activity($-\Delta A_{340} \times 10^2$/min)		Extent of activation (-fold)
		Without ppGpp	With ppGpp	
10	None	4.6	15.8	3.4
2	None	1.5	4.4	2.9
0.2	None	0.07	0.17	2.4
0.2	CoASAc(0.14)	3.5	19.5	5.6
0.2	CoASAc(0.35)	10.5	27.6	2.6
0.2	Palmitoleate (0.1)	2.8	10.0	3.6
0.2	FBP(2.0)	0.18	0.33	1.8
0.2	GTP(1.0)	0.13	0.25	2.0

The constitution of the reaction mixture was the same as described in Materials and methods, except that concentrations of PEP, the effectors and of ppGpp (1.0 mM) were varied as indicated.

was in a range of the intracellular level of PEP in *E. coli* (our unpublished result), ppGpp activated the enzyme synergistically with CoASAc and with palmitoleate by 6-fold and 4-fold, respectively. The extent of activation by ppGpp in the presence of FBP or GTP was lower than that in the absence of each of the effectors. FBP or GTP also activated the enzyme synergistically with CoASAc (not shown). ppGpp seems to activate the enzyme through binding with the enzyme at the same site for GTP presumably owing to their structural similarity.

Figure 1 shows effects of five guanine nucleotides on the activity of PEP carboxylase. The extents of activation in the presence of CoASAc by ppGpp, pppGpp, GTP, and GDP were 9.3-, 3.8-, 5.1- and 6.2-fold,respectively. The respective $A_{0.5}$-values (activation concentration producing half-maximal activation) were 0.5, 0.35, 1.0 and 8.0 mM. GMP was without effect. Among the compounds tested, ppGpp was the most active effector.

Recently, the concentrations of PEP and effectors of PEP carboxylase in *E. coli* cells which were grown

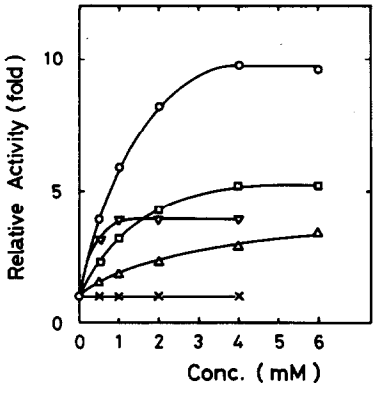

Fig.1. Effects of guanine nucleotides on the activity of PEP carboxylase. The reaction mixture and the assay were the same as described in Materials and methods except for the addition of 0.14 mM CoASAc. (—○—) ppGpp, (—□—) GTP, (—△—) GDP, (—×—) GMP, (—▽—) pppGpp.

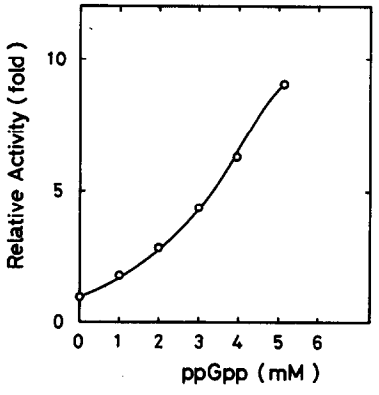

Fig.2. Activation of PEP carboxylase by ppGpp under the 'in vivo' conditions. The reaction mixture was the same as described in Materials and methods except for the addition of 0.35 mM CoASAc, 2.0 mM FBP, 1.0 mM GTP 1.0 mM L-aspartate and of 1.0 mM L-malate.

under various conditions were determined by us. Figure 2 shows the activation of PEP carboxylase by ppGpp in the presence of the same concentrations of PEP and effectors as their intracellular concentrations which were obtained on the cells grown in a medium containing glucose as a sole carbon source (our unpublished results). This indicates that ppGpp is able to activate the enzyme in vivo, since its intracellular concentration is known to attain up to several millimolar in *E. coli* cells. These results led to a speculation that the activity of PEP carboxylase might be regulated under stringent control.

4. Discussion

Until several years ago, stringent control in microorganisms had been thought to exert a negative regulation through the inhibition of biosyntheses of cellular macromolecules. In fact, it had been reported that only the enzymes involved in the biosyntheses were inhibited by ppGpp. Recently, however, ppGpp was reported to stimulate the transcription of *trp* operon [15] or *his* operon [16], suggesting that it is able to play a role also in the positive regulation.

The present investigation indicates that ppGpp was the most active stimulator towards PEP carboxylase of *E. coli* among the guanine nucleotides tested (fig.1). Moreover, the compound was found to be able to activate the enzyme activity not only in vitro but also in vivo (fig.2).

The physiological significance of the activation of PEP carboxylase by ppGpp is postulated as follows: *E. coli* cells respond by controlling their cellular metabolism so as to increase the pools of amino acids when exposed to amino acid deficiency. In fact, it is known under such conditions that the degradation of proteins is stimulated and the transcription of *trp* operon or *his* operon is stimulated by ppGpp [15,16]. The activation of PEP carboxylase results in the increase of oxaloacetate replenishment to the tricarboxylic acid cycle accompanied by the increase of the formations of aspartate and glutamate which are important precursors for other amino acids.

Acknowledgements

We are grateful to Dr Y. Sokawa, Institute for Virus Research, Kyoto University, for discussions and suggestions. This work was supported in part by a grant from the Ministry of Education of Japan.

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