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The Response to the Environmental Stress of Key Enzymes Activities in Central Carbon Metabolic System of *E. coli*

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The response of central carbon metabolism system of *E.coli* to environmental changes is a comparatively simple and meaningful model in the interaction between organisms and environment. We have simulated environmental changes with sodium chloride and paraquat. Analyses of key enzymes activities in branch pathways confirmed the response to environmental stress of central carbon metabolism system of *E.coli* and provided a new visual angle for the regulation mechanism. In 0.2 M NaCl permeability environment, 6-phosphate glucose dehydrogenase/phosphate glucose isomerase revealed significant reduction (~ 69%), the central carbon metabolism flow convert partially from PP pathway to glycolysis pathway. It's just the opposite with oxidative stress situation. Pyruvate kinase and PEP carboxylase is another important adjustable node. The ratio of pyruvate kinase/PEP carboxylase revealed significant reduction (~ 77%) in permeability environment, which is consistent with the decline trend under oxidative stress.

Key words: Central carbon metabolism; Environmental stress; Key enzymes; Regulatory mechanism.

To face varied external environment, cells maintain internal dynamic balance through a series of response to stress[1]. Most of these responses understood were how bacteria adapt to the new environment by adjusting of the number and activity of intracellular proteins through RpoD and RpoS response system[2]. However, from the molecular level, the response to environmental stress is not only the excitation of specific operon but also a complex reaction process which most of genome genes are involved. To discover the relationship and interaction between different function systems under environmental stress, study from the holistic level is preferred. To further clarify the occurrence and development of the whole process of stress, metabolic system is the focus of research because it is both the key component of the environmental stress system and a vital energy source to maintain the survival[3-5].

E.coli is both an important bacteria in the biological industry and an important model organism[6-7]. Study on how the key enzymes in the central carbon metabolism system of *E.coli* response to the environmental stress will not only benefit to the physiology study under environment stress but also help to clarify the reaction and the mechanism of proactive adaption to stress. It provides a new perspective to further understand the interaction between the cell and environment[8].

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Here, we cultivated *E.coli* under the normal, high permeability and oxidative stress respectively. We measured the activity of key enzymes in the branch pathways of central carbon metabolism of *E.coli* and compared the changes of enzyme activity under different conditions. Further determined its migration of carbon metabolic flux under different stress. Metabolic model network was shown in Figure1, while the model can be found in the attachment. Our results show that in the high permeability environment, 6-phosphate glucose dehydrogenase/phosphate glucose isomerase is decreased. And the central carbon metabolism flow convert partially from PP pathway to glycolysis pathway. It's just the opposite situation under oxidative stress. Moreover, the ratio of pyruvate kinase/PEP carboxylase is decreased under high permeability environment, which is consistent with the changes of oxidative stress.

MATERIALS AND METHODS

Cultivation and analyses

E.coli (JM101) strain was used for this research. Frozen stock was inoculated into tubes in 1:1000 dilution and shook overnight at 37 !. When OD grew up to 1.5-2.0, 800 µl broth was harvested and centrifuged 3 minutes at 3500g. Then, plates was washed twice with M9 medium and inoculated to 50 ml control and stressed M9 medium. M9 medium components are as follows: 3 g glucose L^{-1}, 48 mM Na_2HPO_4, 22 mM KH_2PO_4, 10 mM NaCl, and 30 mM $(NH_4)_2SO_4$. 1 mL 1 M $MgSO_4$ solution and 1 mL 0.1 M $CaCl_2$ solution was added in M9 medium per litre [9]. NaCl was added to osmotic stress medium until the final NaCl concentration reached 0.2 M. Paraquat was added to oxidative stress medium until the final paraquat concentration reached 50 mM.

Preparation of crude enzyme solution

When the OD value of culture broth reached 0.8, 50 ml culture was harvested and supernatant removed. Then 10 ml Tris/Mg (pH 8.0) buffer was added to the the plates followed by another wash with Tris/Mg. Cell disruption was performed by sonication. The sonication was set at 5 s brake and 5 s interruption with a total of 70 times. OD600 difference between experiments before and after sonication was used to indicate the crushing efficiency of *E. coli*.

Enzyme activity determination

UV spectrophotometer was preheated 30 min before use. Wavelength was set at 340 nm and 412 nm (the detection wavelength of NADH and NADPH were 340 nm, the detection wavelength of DTNB was 412 nm). Enzymatic reagents were added according to the protocol and placed on 37 ! water bath. Enzyme reaction system was prepared by the foregoing[10-13].

The following enzymes were measured: glucose-6-phosphate dehydrogenase and glucose phosphate isomerase which used Glc6P as substrate, PEP carboxylase kinase and citrate synthetase which used OAA as substrate, isocitrate dehydrogenase which used isocitric acid as substrate and PEP carboxylase which used PEP as substate. For glucose-6-phosphate dehydrogenase, NADPH absorbency was measured. For phosphate glucose isomerase, PEP carboxylase kinase, isocitrate dehydrogenase, isocitrate lyase, PEP carboxylase and pyruvate kinase, NADH absorbency was determined. And for citrate synthase, DTNB absorbency was measured.

RESULTS

Growth curve

E.coli normally grows fast. Experimental data have shown that bacterial strain grew through four stages: lag phase, log phase, stable stage and decline phase. Fig. 1 records the growth curve of several stages. It also shows that the lag phase of normal strain was relatively short while the strain under the environmental stress experienced a relatively long lag phase.

Enzyme activity determination

Table 1 shows that the enzyme activity are significantly different under various culture conditions. It is suggested that more obvious modulation of strains occurred in response to environmental change under different stresses. Figure 2 shows that enzyme activities are all increased at all different levels under oxidative stress compared to the control group of which glucose-6-phosphate dehydrogenate, citrate synthase, PEP carboxylase are increased most significantly. It is suggested that the metabolic flux through the pathways catalyzed by these three

Table 1. The average measurement of key enzymes activities in the various branch pathways

Enzyme name	The average measurements of enzyme activity		
	No environmental stress	Oxidative stress	Salt stress
Glucose-6-phosphate dehydrogenase dehydrogenase	3.79	7.44	4.23
Phosphoglucose isomerase	3.86	5.31	6.22
PEP carboxylase	0.80×10^3	1.32×10^3	1.48×10^3
Pyruvate kinase	0.43×10^3	0.57×10^3	0.61×10^3
Citrate synthase	0.75×10^3	1.20×10^3	1.05×10^3
Isocitric dehydrogenase	4.18	5.37	4.92
Enzyme activity unit: U/L			

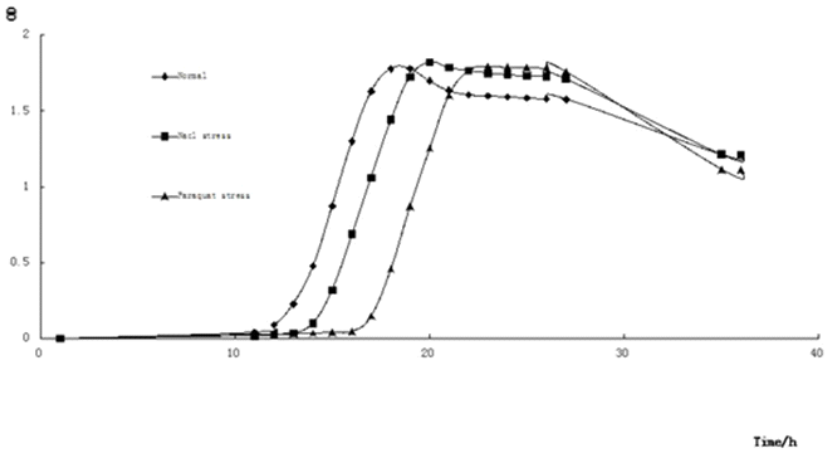

Fig. 1. Growth curves of *E.coli*.The horizontal axis represents time (unit is hour). The vertical axis represents OD_{600}

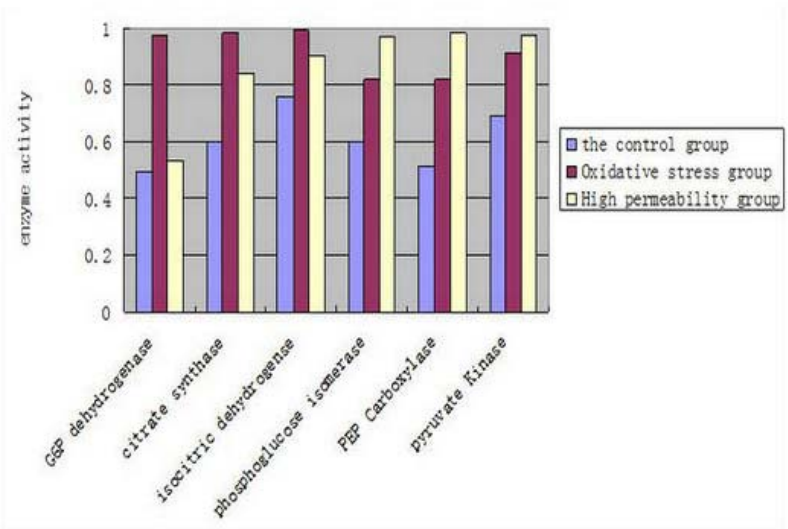

Fig. 2. Comparison of key enzymes activities under different conditions. The abscissa is the ratio of corresponding enzyme activity measured values relative to the maximum value of the three conditions (maximum measured values was 1)

enzymes might be increased significantly compared to the control group. Enzymes activity of high osmotic pressure groups were also increased obviously and the enzyme activity of phosphoglucose isomerase, PEP carboxylase, citrate synthase are increased most significantly. The result suggests that stress conditions have a great influence on the growth of strains and the central carbon metabolism of *E. coli* response to different environmental stress have both commonness and uniqueness.

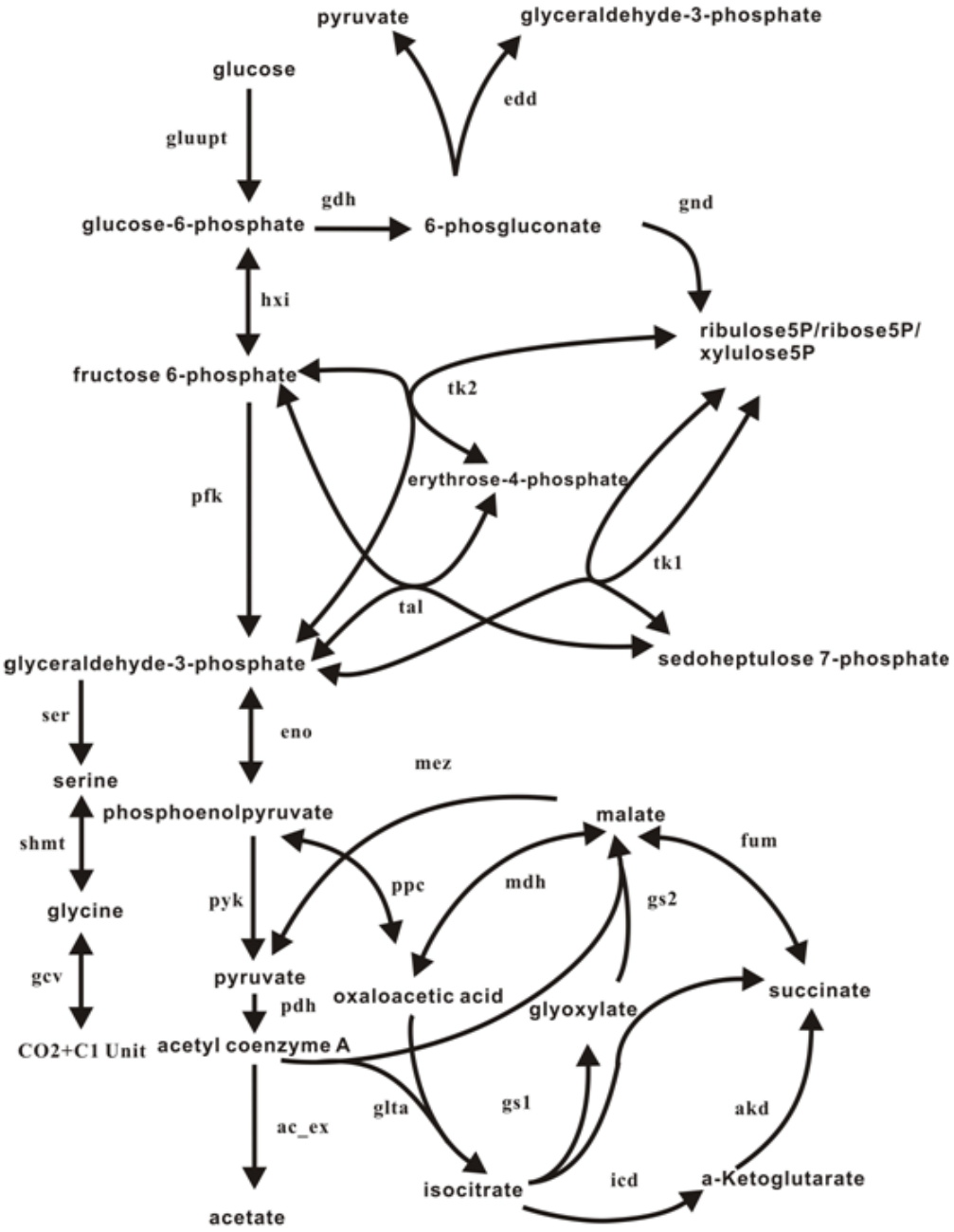

Fig.3 The illustration of central carbon metabolism network of *E.coli* (information came from EcoCyc data base). Reaction direction is indicated by the arrow and the reversible reaction is represented by the double arrow line

DISCUSSION

E. coli can adjust central carbon metabolic pathways accurately to achieve optimal equilibrium growth under oxidative stress.

6-Phosphate glucose dehydrogenase/ phosphate glucose isomerase is increased under oxidative stress. And the flux of central carbon metabolism is converted partly from glycolysis pathway to the PP pathway. There are several effects of PP metabolic pathways: the lose of a carbon atom and provision of necessary reducing power NADPH for the cell growth. At the same time generating all kinds of five carbon sugars which are necessary for cell. Moreover, NADPH is the source of reducing power against oxidative stress. Thus the flux shift from glycolysis pathway to PP pathway is an active choice of *E. coli* to enhance self-protection ability.

The rate of pyruvate kinase/PEP carboxylase is reduced under oxidative stress. Pyruvate kinase is responsible for the step from PEP to PYR. PYR would be oxidized and then a carbon atom is take off. NADH is produced. Then ICIT is produced to supplement the TCA cycle. And PEP carboxylase is responsible for the step from PEP to OAA to supplement the TCA cycle directly. The difference between these two ways is that NADH is produced in the pyruvate kinase pathway. NADH is electron donor for cellular aerobic respiration and has produced reactive oxygen species. Therefore, *E.coli*, reduce its pyruvate kinase and increase PEP carboxylase, it is tantamount to reducing the generation of reactive oxygen species in fact. Consequently, *E. coli* reduce oxidative burden while maintaining the normal TCA cycle. The above results indicate that *E. coli* can control central carbon metabolic pathways accurately to achieve optimal equilibrium growth in response to oxidative stress.

In the high permeability environment, the central carbon metabolism flow convert partially from PP pathway to glycolysis pathway, which is just the opposite with oxidative stress situation.

In as high as 0.2 M NaCl permeability environment, 6-phosphate glucose dehydrogenase/phosphate glucose isomerase is reduced. And the central carbon metabolism flow convert partially from PP pathway to glycolysis pathway. It's just the opposite with oxidative stress situation. Under high permeability environment, cells tend to synthesis glycerol to protect themselves. Due to PP pathway would lose a carbon atom, it is not dominant on the efficiency of carbon conversion to glycerol. The cells enhance glycolytic pathway flow in order to increase the glycerol production efficiency.

The ratio of pyruvate kinase/PEP carboxylase is decreased in high permeability environment, which is consistent with the trend under oxidative stress. From the aspect of the utilization efficiency of carbon atoms, the interests from the PEP carboxylase enter TCA cycle is higher than from the PYR approach. It is possible that cell needs to make full use of the carbon material under high permeability environment.

CONCLUSIONS

The results suggest that *E.coli* will change enzyme activity and metabolic flow of carbon central metabolic pathway to adapt to the environmental changes. Of which, glycolysis and pp pathway as well as pyruvate kinase and PEP carboxylase are two adjustable important nodes. All these adjustments often correspond to certain specific biological purposes. That is to say, when bacteria encounter environmental stress, its system components would be reprogrammed. The reprogramming have a specific biological effect which is ultimately important for the bacteria to adapt to the environmental stress. It biologically aims to reduce the oxidation atmosphere under oxidative stress. This allows bacteria to increase the production of reducing power, including increased NADPH production and decreased NADH production. In the high osmotic pressure environment, the key is on the effective utilization and conversion of carbon atoms as the main purpose of adjusting the flow of carbon metabolism.

The experiments have shown that *E. coli* can take effectively initiative to change carbon center metabolic state to adapt to environmental changes. In future, if research can assemble cell extract experiments, mass spectrometry for the identification of metabolites and their concentration to constitute the flux research experiments on stress mechanism of carbon metabolism of *E. coli*, it will have more significance by making a complete and accurate research on

carbon metabolic regulation mechanism. It is not only beneficial to *E.coli* physiological studies under environmental stress, but also helpful to clarify how *E.coli* carbon center metabolic system reacts on environment stress. The research on mechanism of *E.coli* initiative adapts to environmental stresses provides a new perspective for a thorough understanding the interaction between cells and environment.

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