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CALORIMETRIC STUDIES OF GLYCOLYSIS OF E. COLI UNDER NONGROWING CONDITION

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ABSTRACT

Initial reactions of the metabolic processes in bacterial cells have been investigated by measuring heat generation of E.coli under nongrowing conditions with a titrimetric calorimeter. Three exothermic reactions with different rates of heat generation have been observed calorimetrically under aerobic condition when glucose solution was added to the cell suspension of nongrowing medium. It was estimated that the first exothermic peak obtained after adding glucose was due to uptaking of substrate molecules and following two peaks reflected to the reaction of phosphofructokinase and that of glyceraldehyde-3-phosphate dehydrogenase respectively. To clarify the process of glycolysis, measurements of heat evolution due to metabolisms of various sugars except glucose have also examined and compared with that of glucose.

INTRODUCTION

Calorimetric studies on the growth and metabolisms in bacteria and/or yeasts give us useful informations not only to elucidate the thermodynamical mechanisms of metabolism in vivo, but also to evaluate the effectiveness of energy consumptions in the living cells. The measurement of heat evolution due to metabolic reactions of microbes has been carried out by many authors, and recently fully reviewed (ref. 1 and ref. 2). Many of these, however, are employing the growing cellular systems for their experiments which are including very complex metabolic processes as well as membrane transport of substrates. And yet the most calorimetric experiments are being carried out under isothermal conditions by using batch and/or flow type calorimeters in which it is difficult to control the culture condition during reaction from out side of the reaction vessel by adding substrates or inhibitors and to observe the fast heat evolution processes due to initial metabolic reactions. Therefore, it is still remained as thermodynamically unknown processes of metabolism in the living organs to be fully analyzed the details of each process on the biochemical reaction in vivo.

A study using resting cells of A. aerogenes was reported by Boivinet and Grangetto (ref. 3). By employing nongworing systems to eliminate heat generation due to the anabolic reaction, Nunomura and Fujita also analyzed the heat of endogeneous metabolism and glycolysis of yeast under aerobic condition (ref. 4 and ref. 5). We have already reported the usefulness of titrimetric

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calorimeter for the studies on heat production of microorganisms (ref. 6 and ref. 7). In the present studies, heat of reactions due to glycolysis of E.coli under nongrowing condition have been investigated and thermodynamical mechanisms of the metabolic processes, especially the catabolism of sugars have been analyzed.

MATERIALS AND METHODS

E. coli (IAM 12119) used in this experiment was cultivated in nutrient broth at 310°K under aerobic condition, and harvested in the logarithmic phase of growth. Samples specimen were prepared by washing the cells twice with 0.15 M NaCl aqueous solution and suspending them into phosphate buffer solution(pH 7.0) with a concentration of 0.80 mg cells of dry weight/ml.

Calorimetric measurements were made with a Tronac Model 450 Isoperibol Calori meter (Tronac Inc., Orem, Utah, U.S.A.) employing a 25 ml glass reaction vessel. The principle and methods of this instrument were described elsewhere in detail (ref. 8). 25 ml of cell suspended sample was placed into the reaction vessel and wet air was flowed with a constant rate of 4.5 ml/min at 310°K. After thermal equilibration of the sample, aqueous solution of various sugars with different concentrations was added with a constant volume of 100 ul respectively Heat leakage from the reaction vessel to water bath regulated constant temperature with an accuracy of 10^{-3}K was monitored as a function of time. The heat evolution rate was estimated by the method of electrical calibration.

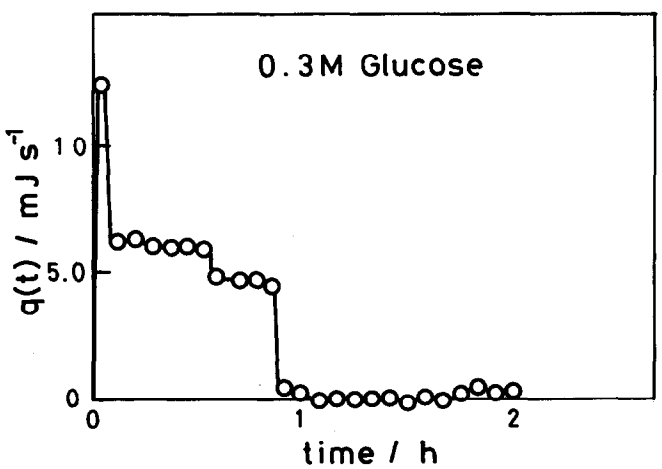
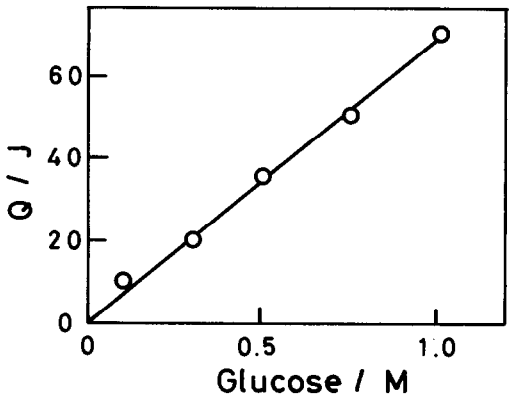


Fig. 1. Heat evolution by glycolysis of E.coli under nongrowing condition. 100 ul of 0.3 M glucose solution was added.



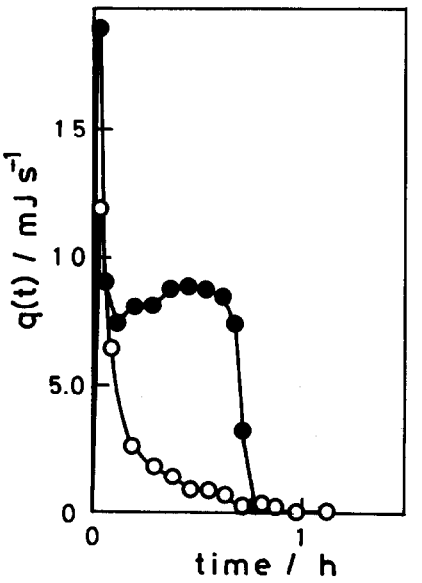


Fig. 2. Effect of glucose concentration. The vertical and horizontal axis show the amount of heat production and concentration of added glucose solution respectively. Each of glucose solution were added in a volume of 100 ul.

Fig. 3. Effect of inhibitors. 25 ul of inhibitor solution was added and three minutes after (at t=0) 100 ul of 0.3 M glucose was added. o——o, 1 mM monoiodoacetic acid(MIAA); •——•, 10 mM carbonyl cyanide m-chlorophenylhydrazone(CCCP).

The amounts of glucose consumption in the reaction vessel were optically measured separately by the use of glucose oxidase method (Glucose C-Test, Wako Chemical Industries, Ltd., Osaka).

RESULTS AND DISCUSSION

The thermal profile obtained by adding glucose to cell suspension of E.coli under nongrowing condition is shown in Fig. 1. It was estimated that the heat evolution due to endogenous metabolism in this system was constant and/or almost absent because the base line of P-T curve was kept constant during no addition of glucose. The heat of reaction due to glucose metabolism of E.coli was observed clearly with three different rate of heat production as shown in Fig. 1, i.e., first reaction exhibiting a short peak obtained after the addition of glucose, second and third reaction exhibiting following two steps. This pattern of heat evolution is very similar with the result of S. cerevisiae observed under nongrowing condition (ref. 5). The glucose concentration in the medium was decreased rapidly and absent during 30 min. after adding of 0.3M glucose in consequence of uptaking by bacterial cells The reaction time of

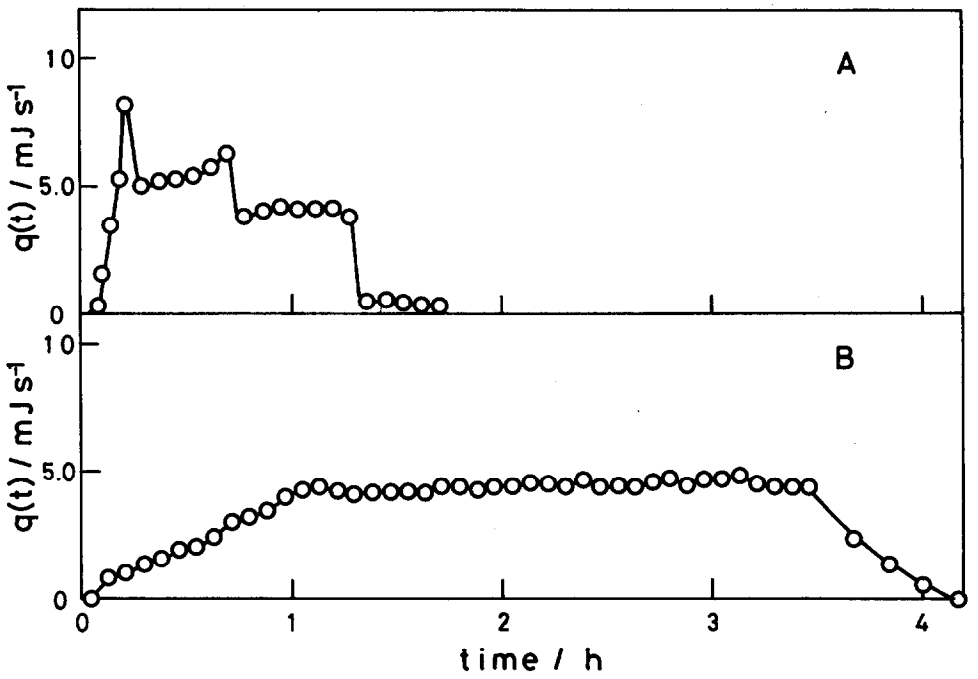


Fig. 4. Heat evolution by other sugars. Each of sugar solution were added in a volume of 100 ul A, 0.3 M fructose; B, 0.3 M glucose-6-phosphate.

second and third steps, on the other hand, were longer than that of uptaking and proportional to the concentration of added glucose. Total amount of heat evolution were also proportional to that of added glucose as shown in Fig. 2. These heat generations were not observed in the case of heat-treated cell suspension of E.coli. This result indicates that the three reactions with different rate of heat evolution are reflecting the catabolic processes of glucose in the cells of E.coli.

In order to elucidate the relationship between metabolic processes and heat evolution, effects of various inhibitors to the heat evolution were investigated. Monoiodoacetic acid(MIAA) is known to inhibit not only the activity of glyceroaldehyde-3-phosphate dehydrogenase in glycolysis cycles under low concentration, but also the uptaking of glucose under higher concentration. Therefore, effect of MIAA was examined and the result obtained was shown in Fig. 3. The result of this experiment indicates clearly that addition of low concentration of MIAA is completely inhibiting the second and third reaction shown in Fig. 1, but the

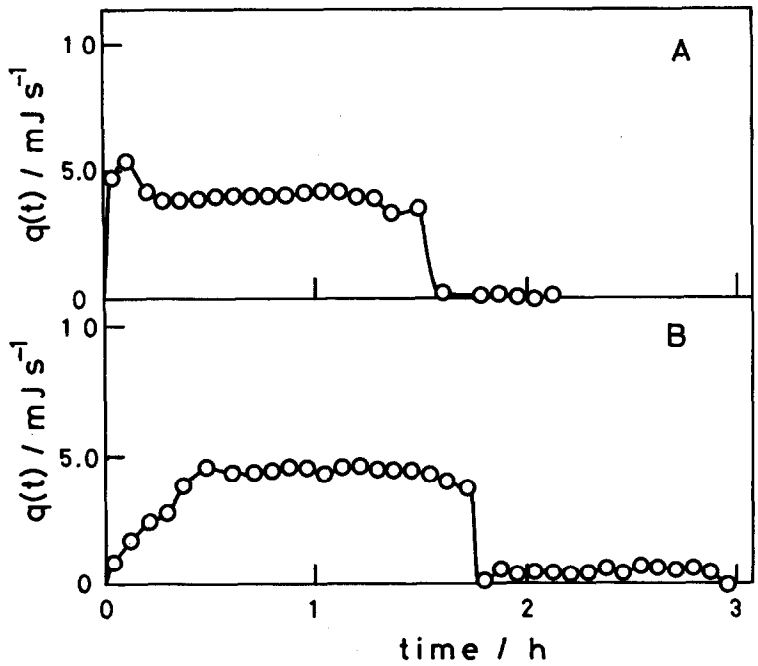


Fig. 5. Heat evolution by other sugars. Each of sugar solution were added in a volume of 100 ul. A, 0.3 M mannose; B, 0.3 M galactose.

uptaking of glucose is not still inhibited (Fig. 3). Under high concentration of MIAA, no heat evolution was detected, that is indicating inhibition of the uptaking of glucose. These findings suggest us that the first reaction exhibited with a narrow peak is due to the uptaking of glucose, the second and third reactions reflect the following processes which are glycolysis and respiratory reactions in the living cells. As shown in Fig. 3, carbonyl cyanide m-chlorophenyl hydrazone (CCCP) which inhibits the oxidative phosphorelation also influenced on the second and third reactions but the pattern of P-T curve was just different from the case of MIAA.

To make the pathway of glycolysis more clear calorimetrically, heat productions due to metabolism of fructose, glucose-6-phosphate, mannose, and galactose by E.coli have also observed as shown in Fig. 4 and Fig. 5. The pattern indicating changes of heat evolution rate vs. cultivation time was different each other, but in the case of fructose the thermal profile was similar with that of glucose (A in Fig. 4). Differences of P-T curve among sugars used in this experiment are indicating that the pathway of catabolic processes and uptaking of these sugars are different each other. However, total amounts of

heat evolved among these sugars were almost equal except that of glucose-6-phosphate.

According to the studies on the sugar uptaking and its transport in microbial cells, it is known that uptakes of glucose, fructose, and mannose in E.coli are carried out by the phosphotransferase while glucose-6-phosphate and galactose uptake involve other processes participating permeases (ref. 9).

As a matter of fact, the P-T curves obtained by glucose was very similar in the first peak indicating uptake of sugars with that of fructose and mannose while glucose-6-phosphate and galactose showed no distinct narrow peak in their curves of heat evolution.

Detailed mechanisms of energetics coupled with sugar transport have still remained unclear, however, the results in our experiments indicate that differences of the first peak in heat evolution curves reflect the different mechanisms for uptaking process and/or transport of sugars as described above.

Calorimetric studies on the metabolism of microorganism thus give us very useful information to elucidate the mechanisms of sugar transport as well as energetics of glycolysis. Quantitative analysis of our experiment is now in progress.

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