EVIDENCE THAT ATP EXERTS CONTROL OF THE RATE OF GLUCOSE UTILIZATION IN THE INTACT Escherichia coli CELL BY ALTERING THE CELLULAR LEVEL OF GLUCOSE-6-P, AN INTERMEDIATE KNOWN TO INHIBIT GLUCOSE TRANSPORT In Vitro

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SUMMARY. The effects of treating nitrogen-starved cultures of Escherichia coli W4597(K) with various doses of 2,4-dinitrophenol include increases in the rates of glucose utilization, decreases in ATP and glucose-6-P and maintenance of the level of fructose-1,6-P_2. A quantitative correlation was observed between the increases in the rates of glucose utilization and decreases in glucose-6-P in agreement with the observation made in vitro that glucose-6-P inhibits glucose transport in E. coli. A quantitative correlation was also observed between glucose-6-P and ATP indicating that the fall in glucose-6-P is effected by the fall in ATP which indirectly signals increased glucose utilization and increased ATP production.

Bovell and Helgerson (1) reported in an abstract that DNP[1] inhibited glycogen accumulation by nitrogen-starved E. coli ML30; however, no detailed studies to this effect have been reported. We undertook to study this effect in order to further strengthen the quantitative correlation we have reported (2,3) between effectors of ADPG synthetase and the rate of glycogen synthesis in the intact E. coli cell. Correlation was observed and will be reported in a future communication. In order to establish that alterations in glycogen synthesis were not the result of inhibition of glucose utilization we studied the effect of various doses of DNP on the rate of glucose utilization in nitrogen-starved E. coli W4597(K) in the presence of excess glucose. The results of this study are presented in this report.

As the dose of DNP increased the rate of glucose utilization increased while the cellular levels of G6P and ATP decreased and the cellular level of FDP was not affected. A quantitative correlation was observed between increases

[1]Abbreviations used: DNP, 2,4-dinitrophenol; FDP, fructose-1,6-P_2; F6P, fructose-6-P; G1P, glucose-1-P; G6P, glucose-6-P; PFK, phosphofructokinase.

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in the rates of glucose utilization and decreases in the level of G6P indicating that G6P is an inhibitor of glucose utilization in the intact E. coli cell, in agreement with the observation made in vitro that G6P inhibits glucose transport in isolated E. coli membrane preparations (4). A quantitative correlation was also observed between the cellular levels of G6P and ATP suggesting that the decrease in G6P is effected by the decrease in ATP. It appears that under these conditions a decrease in ATP indirectly signals increased utilization of glucose, a process which would lead to increased ATP production.

EXPERIMENTAL. E. coli W4597(K) is a uridine diphosphate glucose pyrophosphorylase negative derivative of E. coli K12. The cells were cultured aerobically at 32°C in a synthetic medium containing 4.0 g D-glucose per liter; medium ammonium nitrogen (limiting nutrient), sulfate, phosphate and minerals were maintained at the levels previously reported (5,6). The cultures were inoculated from a Trypticase Soy Broth (Baltimore Biological Laboratories) suspension and were grown for approximately 18 hrs until medium nitrogen had been exhausted. At the onset of nitrogen depletion the culture contained 188 mg protein per liter and the optical density at 450 nm (1 cm light path) was 1.25.

Fifteen minutes after the depletion of exogenous nitrogen aliquots were removed to flasks containing the appropriate amounts of DNP and the flasks were returned to the gyrotory incubator operating at 316 cycles/min with a 2.5 cm amplitude. At 0.25 hr after dividing the culture and at various times during the next 2.75 hrs aliquots were taken from each of the flasks and prepared for metabolite and glucose measurements as previously described (5,6).

Glucose, ATP, FDP and G6P measurements were performed and the rates of glucose utilization were calculated as previously described (5,6).

RESULTS AND DISCUSSION. The steady-state levels of ATP, FDP and G6P and the constant rates of glucose utilization observed over a 2.75 hr period in nitrogen-starved E. coli W4597(K) in the presence of various concentrations of DNP are summarized in Table I. The cellular rates of glucose utilization increased and ATP and G6P decreased in response to increasing concentrations of DNP. The cellular level of FDP remained constant throughout these experiments. A plot of the reciprocals of the rates of glucose utilization on the corresponding cellular levels of G6P yields a straight line with a correlation coefficient of 0.999 (Fig. 1), and is typical of plots obtained from studies of isolated enzymes performed in vitro where the concentration of an inhibitor is varied and the substrate is held constant (7).

G6P is a noncompetitive inhibitor of glucose transport in isolated membrane preparations from E. coli and a much more effective inhibitor than G1P or F6P,

Table I. Effect of 2,4-Dinitrophenol on the Rates of Glucose Utilization, ATP, G6P and FDP in Nitrogen Starved E. coli W4597(K)

DNP mmoles/l	Glucose Utilization mg/mg prot/hr	G6P µmoles/g prot.	ATP µmoles/g prot.	FDP µmoles/g prot.
None	0.54	5.29 (0.70)	10.5 (0.55)	4.27 (0.39)
None	0.54	5.21 (0.20)	10.1 (1.14)	4.34 (0.28)
0.17	0.75	3.61 (0.54)	9.28 (0.76)	4.30 (0.21)
0.25	0.94	2.68 (0.34)	8.07 (0.61)	4.47 (0.38)
0.33	1.07	2.16 (0.35)	7.21 (0.90)	4.31 (0.40)
0.50	1.55	1.23 (0.28)	5.92 (0.52)	4.29 (0.71)

The growth of the organism, composition of the medium, glucose and metabolite assays are the same as those indicated in the text. The rates of glucose utilization are expressed as mg glucose utilized per mg protein per hour. Metabolites are expressed as µmoles per g protein; each value represents the mean of five determinations and the standard deviation is contained in parentheses.

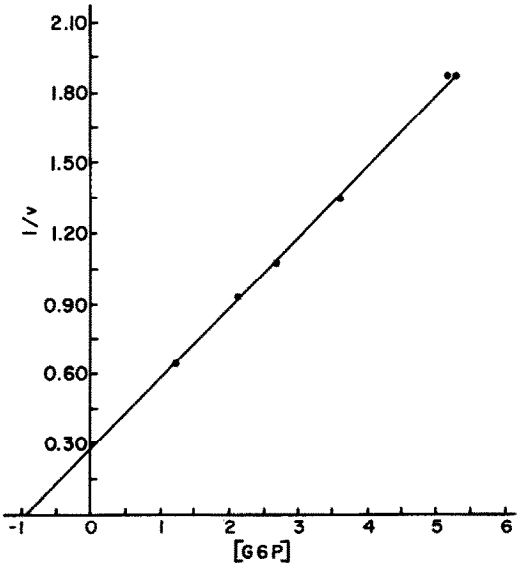

Fig. 1 Relationship of the reciprocal of the rate of glucose utilization and the cellular level of G6P in the presence of various concentrations of DNP. The rates of glucose utilization (v) and the concentrations of G6P were taken from Table I and are expressed in the same units. The line was obtained from a linear regression analysis and a correlation coefficient of 0.999 was obtained.

intermediates which would be expected to covary with G6P *in vivo* (8). Glucose transport in membranes from glucose-grown cells is inhibited 75% by 1 mM G6P and 15% by G1P at concentrations of 1 mM and higher (4). While the effect of F6P on glucose transport in membranes from glucose-grown cells was not reported, 5 mM F6P inhibited glucose transport in membranes from succinate-grown cells only about one-fifth as much as did 5 mM G6P (4). Assuming 5 ml cell water per g protein (3), the intracellular concentration of G6P reported here in the absence of DNP is approximately 1 mM, a concentration which significantly inhibited glucose transport in the study of isolated membrane preparations cited above. The intracellular concentration of G1P in *E. coli* has been estimated to be 0.02 mM (4,9) and that of F6P in glucose-NH_4 grown cells to be 0.3 mM (9), each concentrations which would have produced far less significant inhibition of glucose transport *in vitro* than did physiological concentrations of G6P. It would appear therefore, that the quantitative correlation reported here between decreases in the steady-state level of G6P and increases in the constant cellular rates of glucose utilization represents the inhibition of glucose transport by hexose phosphates and that in this case the most effective inhibitor is G6P.

Unlike studies performed *in vitro* where the investigator can alter the variables at will it is not possible to determine from these *in vivo* data if G6P is acting as a competitive or noncompetitive inhibitor. However, it is noteworthy that the $I_{0.5}$ (the amount of inhibitor at which 50% inhibition occurs) obtained for the noncompetitive inhibition by G6P of glucose transport by membranes from glucose-grown cells is 0.5 mM (4) which is within the range of G6P levels reported here (0.25 to 1.05 mM) and which is similar to the negative intercept of the abscissa in Fig. 1 (0.18 mM). In cases of noncompetitive inhibition the value of this intercept is equal to the $I_{0.5}$ (7).

A reciprocal plot of the cellular levels of G6P on the square of the level of ATP yields a straight line with a correlation coefficient of 0.998 suggesting a causal relationship between the two parameters (Fig. 2). The link between ATP and G6P may be that a decrease in ATP, an allosteric inhibitor of *E.*

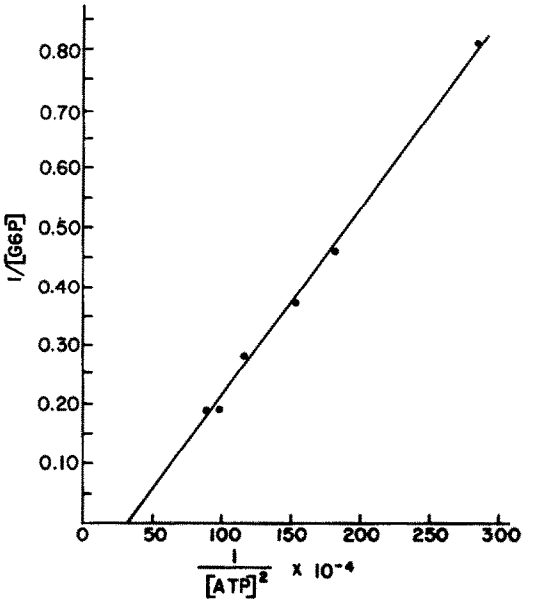

Fig. 2. Relationship of the reciprocals of the cellular level of G6P and of the square of the cellular level of ATP in the presence of various concentrations of DNP. The concentrations of G6P and ATP were taken from Table I and are expressed as μmoles per g protein. The line was obtained from a linear regression analysis and a correlation coefficient of 0.998 was obtained.

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coli PFK in vitro (10-12), results in increased PFK activity, draining the supply of G6P. The lower steady-state levels of G6P established with increasingly higher rates of glucose utilization indicate that under these conditions the rate of formation of G6P (the transformation of medium glucose to intracellular G6P) is less than the rate at which G6P is metabolized via PFK.[2]

A decrease in ATP and a resultant increase in PFK activity would be expected to result in an increased level of FDP (13); however, in these experimental conditions the level of FDP remained constant. Apparently some other process compensates for increased FDP production to maintain FDP at a constant level. Such a mechanism would be useful to a cell which is being challenged by a wasteful drainage of ATP. A rise in FDP would lead to an increase in glycogen synthesis (2,14), shunting G6P away from glycolysis, an ATP producing pathway, and

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[2]The percent of glucose converted into glycogen decreases with increasing concentrations of DNP from a control value of 20% of the glucose used by the cell. The activity of the hexose monophosphate shunt is low in resting E. coli (15).

into glycogenesis, an ATP consuming pathway. Maintenance of the level of FDP would insure that the increased supply of glucose would be used for ATP production. Furthermore, in studies to be presented elsewhere, a decrease in cellular G6P in this organism is paralleled by a decrease in glycogen synthesis, a process which would yield an even more efficient shunting of the increased glucose supply into glycolysis. Evidently the controls on the interactions of these various processes are finely tuned since a fall in FDP under these conditions would be deleterious to the cell because, as we have previously demonstrated (6), a decrease in FDP is paralleled by decreased utilization of glucose.

Apparently a different mechanism is operative in the presence of an overflow of energy, for example, in the transition from exponential growth to a nutrient-limited stationary phase (2,3,5,6). In these cases an increase in ATP resulting from a decreased demand for ATP in net protein and nucleic acid synthesis is accompanied by decreases in FDP and the level of G6P remains constant. Based on a previous study (6) a fall in FDP in the presence of a constant level of G6P would be expected to result in a decreased rate of glucose utilization. The increase in ATP offsets the decreased FDP resulting in an increased synthesis of glycogen (2,3). Thus it appears that the cell faced with an excess supply of energy decreases its overall utilization of glucose and shunts more of the available glucose into an energy storage pathway and away from an energy producing pathway. Why this mechanism (decreasing FDP with constant G6P) would be elicited in nutrient starvation rather than a reversal of the mechanism operative in response to a challenge by DNP (decreasing G6P with constant FDP) is not clear at present. Hopefully further study will elucidate how the same signal, changes in the cellular level of ATP, can effect two different metabolic responses, each apparently useful to the cell.

The data in the preceding report on the covariance of FDP and the rate of glucose utilization (6) predict a rate of 0.77 mg glucose utilized/mg protein/hr in the presence of 3.6 μmoles G6P/g protein and 4.3 μmoles FDP/g protein. At the same levels of FDP and G6P in this report, in the presence of 0.17 mM DNP,

the observed rate of glucose utilization is 0.75 in good agreement with the predicted rate demonstrating the consistancy of the data obtained from two widely differing experimental approaches. This good agreement is consistent with the interpretation that the cellular levels of FDP and G6P, or metabolites which covary with them, regulate the rate of glucose utilization in E. coli.

The quantitative correlation between G6P and the rate of glucose utilization reported here is the first evidence that G6P regulates the rate of glucose utilization in vivo.

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