MAINTENANCE OF THE ENERGY CHARGE IN THE PRESENCE OF LARGE DECREASES IN THE TOTAL ADENYLATE POOL OF Escherichia coli AND CONCURRENT CHANGES IN GLUCOSE-6-P, FRUCTOSE-P_2 AND GLYCOGEN SYNTHESIS[1]

David N. Dietzler, Cynthia J. Lais, John L. Magnani, and Mary P. Leckie

Departments of Pediatrics and Pharmacology
Washington University School of Medicine
St. Louis, Missouri 63110

Received August 8,1974

SUMMARY. Lowered aeration and lowered aeration in the presence of chloramphenicol cause a respective 40% and 60% decrease in the total adenylate concentration of nitrogen-starved E. coli W4597(K). These decreases are accompanied by maintenance of the adenylate energy charge and adenylate kinase mass action ratio, increases in fructose-1,6-P_2 and glucose-6-P, and decreases in the net rates of glycogen synthesis. The important new observation is that a drastic decrease in the total adenylate pool is accompanied by complete maintenance of the energy charge. These decreases in the adenylate pool are correlated with the accompanying metabolic changes.

Metabolic stress can result in a decrease in the total concentration of adenine nucleotides with only a small decrease in the adenylate energy charge (1-6). Apparently when cellular ATP decreases an enzyme which destroys AMP is activated (7,8) and the relative concentrations of the adenine nucleotides are restored through the action of adenylate kinase preserving the energy charge at the expense of the adenine nucleotide pool. For example, treatment of fetal rat heart with chloramphenicol (5) results in a decrease in ATP and this decrease is accompanied by a decrease in the adenine nucleotide pool, maintenance of the adenylate kinase mass action ratio and a drop in energy charge from 0.88 to 0.84. A loss of ATP with maintenance of the total pool would have resulted in an energy charge value of 0.76.

In one of the studies presented in this report if the pool had been maintained in the presence of the observed fall in ATP the energy charge would

---

[1]This research was supported by Public Health Service Grant AM10766 and a grant from the St. Louis Children's Hospital.

have fallen from an initial value of 0.87 to less than 0.50. Instead as the result of the accompanying decrease in the total pool the energy charge was maintained at the initial value.

This study is the first report of a large decrease in the adenylate pool accompanied by complete maintenance of the energy charge. These decreases in cellular adenylates in nitrogen-starved E. coli W4597(K) subjected to decreased aeration and chloramphenicol treatment was accompanied by increases in FDP[2] and G6P and decreases in the net rate of glycogen synthesis. The decreases in the adenine nucleotide pool are correlated with the accompanying metabolic changes.

EXPERIMENTAL. Culture techniques and glycogen, adenine nucleotides, FDP and G6P measurements were performed and the rates of glycogen synthesis were calculated as previously described (9).

The characteristics of the exponential phase of growth in a culture of this type have been described in detail (9,10). At the onset of nitrogen depletion in the presence of an excess of glucose the optical density of the culture (1 cm light path) at 450 nm was 1.22, corresponding to 183 mg protein per liter of culture and 7.3×10^8 cells per ml (9). Fifteen minutes after the depletion of medium nitrogen three 100-ml aliquots were removed to 250-ml Erlenmeyer flasks, one of which contained 500 mg CM per liter. One of the aliquots which contained no CM was returned to the gyrotory shaker operating at 316 cycles/min (2.5 cm amplitude) while the other two aliquots were incubated in another gyrotory shaker operating at 114 cycles/min (the lowest speed attainable).

At 0.25 hours after dividing the culture and at various times during the next 2.5 hrs aliquots were taken from each of the flasks and prepared for metabolite and glycogen measurement as previously described (9). Aliquots for the measurement of pO_2 (5 ml) were drawn into a glass syringe containing 0.1 ml 40% formaldehyde. The pO_2 of the sample was then measured using a Corning model 160 blood gas analyzer operating at 37°C.

RESULTS AND DISCUSSION. In all of the culture aliquots whether containing no CM and agitated at either 316 or 114 cycles/min or containing 500 mg CM/l and incubated at the slower rate of agitation the viable cell count and the concentration of protein in the cultures observed at the onset of nitrogen depletion were maintained throughout the course of the experiments. The pO_2 of the culture maintained at the higher rate of agitation was 132 ± 5.0 mm Hg (mean and standard deviation of six measurements during the course of the experi-

---

[2]Abbreviations used: FDP, fructose-1,6-P_2; G6P, glucose-6-P; G1P, glucose-1-P; CM, chloramphenicol; P_i, inorganic phosphate.

Table I

Effects of Low Aeration and Chloramphenicol (CM) on the Levels of Adenine Nucleotides and the Values of Their Derived Parameters and on the Rate of Glycogen Synthesis and the Levels of FDP and G6P in Nitrogen-Starved *E. coli* W4597(K).

Parameter	Experimental Conditions		
	High Aeration	Low Aeration	Low Aeration + 500 mg CM/l
ATP	11.50 (0.26)	7.24 (0.48)	4.49 (0.40)
ADP	3.09 (0.16)	1.52 (0.21)	1.21 (0.13)
AMP	0.57 (0.03)	0.22 (0.01)	0.22 (0.03)
Total Adenylates	15.18 (0.33)	8.98 (0.48)	5.93 (0.55)
Mass Action Ratio	0.69 (0.07)	0.72 (0.23)	0.68 (0.09)
Energy Charge	0.86 (0.01)	0.89 (0.01)	0.86 (0.01)
Glycogen Synthesis	468	326	182
FDP	3.75 (0.16)	6.52 (0.41)	11.40 (0.75)
G-6-P	3.93 (0.30)	4.69 (0.18)	6.01 (0.52)

High and low aeration refer respectively to agitation of the cultures at 316 or 114 cycles/min. The composition of the medium, glycogen and metabolite assays are the same as those indicated in the text. Metabolites are expressed as μmoles/g protein; each value represents the mean of values determined for five different samples collected at intervals throughout a three hr period. The rate of glycogen synthesis is expressed as μmoles of glycogen glucose/g protein/hr. The adenylate kinase mass action ratios, $(ATP)(AMP)/(ADP)^2$, and energy charge values, (ATP) + 1/2 (ADP) divided by total adenylates, are unitless parameters. The values contained in the parentheses are the standard deviations.

ment) while the pO_2 of the cultures agitated at the slower speed was 21 ± 4.5. Uninoculated media agitated at either rate maintained a pO_2 of 161 ± 2.9.

In each of the cultures the levels of adenine nucleotides, FDP and G6P remained constant throughout the course of the experiment (Table I). Differences between individual measurements of a given parameter in each of the cultures are within the experimental error of the analytical procedures. The levels of the adenine nucleotides, FDP, G6P and the rate of glycogen synthesis in the culture maintained at the higher rate of agitation are in agreement with

results we have previously presented for these conditions (9-11). In both cultures agitated at the slower rate in the presence or absence of CM the constant levels of adenine nucleotides were lower and the constant levels of FDP and G6P were higher than those in the untreated culture maintained at 316 cycles/min. None of these changes are the result of a decreased utilization of glucose. Other experiments not reported here have demonstrated an increased rate of glucose utilization in nitrogen-starved cultures agitated at 114 cycles/min; CM does not decrease this increased rate or may increase it very slightly.

Although the total concentration of adenine nucleotides decreased 40% in the culture agitated at the slower rate and 60% when CM was added, the energy charge and the adenylate kinase mass action ratio were in each case maintained (Table I). As the adenine nucleotide pool drops, ATP decreases and FDP rises consistent with the observation that ATP inhibits E. coli phosphofructokinase (12-14).

In each of the three conditions the cellular level of glycogen increases linearly throughout the course of the experiment (Fig. 1). In each case the slope of the line represents the constant net rate of glycogen synthesis and is proportional to the rate per cell (9). These slopes were obtained by linear regressional analysis and in each case demonstrate a correlation coefficient greater than 0.995. The net rate of glycogen synthesis decreased in the culture agitated more slowly and with the addition of CM decreased even further (Table I). These decreases are paralleled by decreases in the total adenylate pool (with maintenance of the energy charge) and increases in FDP and G6P. An increase in FDP and in G6P, which presumably reflects a parallel change in G1P, at a constant energy charge value would be expected to effect an increase in the rate of glycogen synthesis (11,15). Presumably this increase does occur but is offset by a greater increase in glycogen degradation resulting in a decrease in the observed net rate. The parallel increases in G6P may reflect this proposed increase in glycogen degradation.

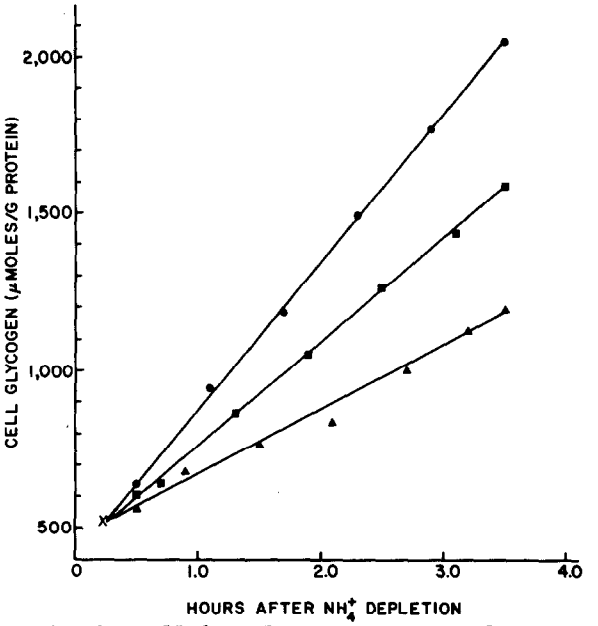

Figure 1. Changes in the cellular glycogen content of E. coli W4597(K) during nitrogen starvation in cells aerated by agitation in a gyrotory incubator with a 2.5 cm amplitude at 316 cycles per minute (●---●), 114 cycles per minute (■---■), and at 114 cycles per minute in the presence of 500 mg CM/l (▲---▲). The X point represents the glycogen level present at the time aliquots were withdrawn from the working culture. The rates of glycogen synthesis (μmoles glycogen glucose incorporation/g protein/hr) were obtained by evaluation of the slopes of the regression lines for the cellular glycogen level versus time for each experiment. These rates are proportional to the rates per cell (9). Culture techniques and glycogen measurements were performed as outlined in the text.

If the phosphate in the adenine nucleotides removed from the pool was released to form P_i then an increase in intracellular P_i would be expected to accompany the decrease in the adenylate pool, possibly stimulating the activity of glycogen phosphorylase. Other investigators have predicted that P_i would increase as the adenylate pool decreases (7). The decreases in the net rates of glycogen synthesis reported here which parallel decreases in the adenylate pool may be indirect evidence for a rise in intracellular P_i.

As in experiments reported here bacteria are conventionally grown in the presence of high concentration of medium phosphate rending measurement of intracellular P_i impossible and thus no data are available on the P_i content of bacteria. The initial studies presented here provide a sound basis for an attempt to reproduce these experiments using a culture medium with a low con-

centration of phosphate. Hopefully the change in medium would not alter the observed metabolic responses and the additional measurement of intracellular P_i could be achieved providing further information concerning the validity of the proposed metabolic interactions.

In summary we have proposed that the activity of phosphofructokinase increases as a result of the observed drop in ATP, resulting in the observed rise in FDP and that an increase in intracellular P_i which may accompany the observed decrease in the adenylate pool stimulates glycogen degradation, increasing G6P. Although the rise in FDP and G6P in the presence of a constant energy charge would lead to an increase in the rate of glycogen synthesis, we propose that this increased rate is offset by a greater increase in degradation resulting in a decrease in the net rate of glycogen synthesis.

The inhibition by CM of glycogen accumulation by nitrogen-starved E. coli W4597(K) has previously been demonstrated in another laboratory (16). These same investigators also observed that CM caused an increase in glycogen accumulation by exponentially growing bacteria (17). In order to explain these contradictory effects they postulated that the increase in protein turnover which occurs in nutrient limitation of bacteria causes the loss of an enzyme necessary for glycogen synthesis and that CM inhibits the enzyme's resysthesis[3] (18). Two observations argue against this mechanism. First of all, CM not only inhibits protein synthesis in E. coli, it also blocks the increased turnover of protein (19). Secondly, the change in the rate of glycogen synthesis which we observed in the CM-treated culture seems to occur more rapidly than can be accounted for by the loss of an enzyme. Detailed studies on the effect of CM on the rate of glycogen synthesis are in progress.

In any case, the important new observation presented here is that a drastic decrease in the total adenylate pool is accompanied by complete maintenance of the energy charge in E. coli and the accompanying metabolic changes appear to be consistent with these changes in cellular adenylates. It is

[3]CM is not preventing an increase in the level of a synthetic enzyme since the levels of these enzymes do not change at the transition from exponential growth to the nitrogen-limited stationary phase in E. coli W4597(K) (9).

interesting to note that if in the culture agitated at the slower velocity and treated with CM the observed decrease in ATP had been accompanied by maintenance of the adenylate pool the value of the energy charge would have fallen below 0.50. Below this value E. coli can no longer maintain viability (6). Thus, the buffering effect of a decreased adenine nucleotide pool is important to the survival of the cell in a hostile environment.

REFERENCES

1. Burch, H.B., Lowry, O.H., Meinhardt, M.P., Jr., and Chyu, K. (1970) J. Biol. Chem. 245, 2092-2101.
2. Mäenpää, P.H., Raivio, K.O., and Kekomäki, M.P. (1968) Science 161, 1253-1254.
3. Raivio, K.O., Kekomäki, M.P., and Mäenpää, P.H. (1969) Biochem. Pharmacol. 18, 2615-2624.
4. Woods, H.F., Eggleston, L.V., and Krebs, H.A. (1970) Biochem. J. 119, 501-510.
5. Hallman, M. (1973) Pediat. Res. 7, 923-930.
6. Chapman, A.G., Fall, L., and Atkinson, D.E. (1971) J. Bacteriol. 108, 1072-1086.
7. Chapman, A.G., and Atkinson, D.E. (1973) J. Biol. Chem. 248, 8309-8312.
8. Schramm, V.L., and Leung, H. (1973) J. Biol. Chem. 248, 8313-8315.
9. Dietzler, D.N., Leckie, M.P., and Lais, C.J. (1973) Arch. Biochem. Biophys. 156, 684-693.
10. Dietzler, D.N., Lais, C.J., and Leckie, M.P. (1974) Arch. Biochem. Biophys. 160, 14-25.
11. Dietzler, D.N., Leckie, M.P., Lais, C.J., and Magnani, J.L. (1974) Arch. Biochem. Biophys. 162, 602-606.
12. Blangy, D., Buc, H., and Monod, J. (1968) J. Molec. Biol. 31, 13-35.
13. Lowry, O.H., and Passonneau, J.V. (1964) Naunyn-Schmiedbergs Arch. Exp. Pathol. Pharmakol. 248, 185-194.
14. Atkinson, D.E., and Walton, G.M. (1965) J. Biol. Chem. 240, 757-763.
15. Govons, S., Gentner, N., Greenberg, E., and Preiss, J. (1973) J. Biol. Chem. 248, 1731-1740.
16. Sigal, N., Cattaneo, J., and Segel, I.H. (1964) Arch. Biochem. Biophys. 108, 440-451.
17. Segel, I.H., Cattaneo, J., and Sigal, N. (1963) in Proceedings of the International C.N.R.S. Symposium on the Mechanism of Regulation of Cellular Activities in Microorganisms, p. 337, Centre National de la Recherche Scientifique, Paris.
18. Cattaneo, J., Sigal, N., Favard, A., and Segel, I.H. (1966) Bull. Soc. Chim. Biol. 48, 441-445.
19. Pine, M.J. (1973) J. Bacteriol. 115, 107-116.