Relation of Growth and Protein Synthesis to the Adenylate Energy Charge in an Adenine-requiring Mutant of *Escherichia coli*

JEAN S. SWEDES,‡ ROBERT J. SEDO, AND DANIEL E. ATKINSON

From the Molecular Biology Institute and the Biochemistry Division of the Department of Chemistry, University of California, Los Angeles, California 90024

When *Escherichia coli* K-12 strain PC 0294, which is unable to synthesize adenine nucleotides, threonine, proline, and leucine, was starved for adenine, the concentrations of ATP, ADP, and AMP fell rapidly. The adenylate energy charge was not affected until the adenine nucleotide pool fell to about 30% of its normal value. Similarly, cells in an adenine-limited chemostat grew at intracellular adenine nucleotide pool values as low as 30% of normal, but the energy charge in all cases was approximately 0.90. Incorporation of [¹⁴C]leucine into protein in adenine-starved cells continued rapidly but the concentration of ATP fell, presumably because of nucleic acid synthesis. The rate of [¹⁴C]leucine incorporation into protein rose rapidly while adenine nucleotide concentrations fell, and then declined roughly in parallel with the energy charge. At a given value of energy and of total concentration of adenine nucleotides, the rate of protein synthesis may, of course, vary with the concentrations of precursors and modifiers; thus, the rate cannot be predicted from knowledge only of the energy charge. Our results suggest, however, that the rate of protein synthesis and the capacity for growth are much more sensitive to changes in the value of the energy charge than to changes in the concentration of ATP. Growth occurs when the ATP concentration is reduced to one-third of its normal value, but has not been observed when the energy charge has fallen by as much as 10%.

The adenine nucleotides stoichiometrically couple energy-producing and energy-utilizing metabolic sequences. The adenylate energy charge, defined as \([\frac{[ATP]}{[ATP] + [ADP] + [AMP]}]\), is a linear measure of the amount of metabolic energy stored in the adenine nucleotide pool. Studies in vitro have shown that the catalytic properties of enzymes from both catabolic and biosynthetic sequences are modified by changes in the energy charge (1-8). Studies in vivo have shown that the energy charge is stabilized in the range 0.8 to 0.95 in normal cells (9) as predicted from the kinetic studies (10). When cells are subjected to metabolic stress such as limitation in their energy source, nitrogen source, or phosphate supply, or a sudden increase in the concentration of a phosphoryl acceptor, there is usually only a moderate change in the energy charge, indicating that it is a tightly controlled parameter (9, 11-23).

Enzymes that show a response to the value of the energy charge in vitro have been found to be insensitive to variations in the total adenine nucleotide concentration (and thus to the concentration of ATP), within the physiological range of adenylate concentrations, the activity of the enzyme depends on ratios of the nucleotide concentrations (the energy charge) rather than on absolute concentrations (see under "Discussion" for references). These observations strongly support the prediction, based on the metabolic roles of the adenine nucleotides as coupling agents between metabolic sequences, that the energy charge rather than absolute concentrations should be the effective regulatory parameter in vivo. This prediction is difficult to test directly because these variables tend to rise and fall together in the intact cell. In this paper, we report that this covariance of the ATP level and the energy charge can be partially uncoupled by adenine limitation of an adenine auxotroph of *Escherichia coli*. The results suggest that the rate of protein synthesis and the capacity for growth are more closely related to the energy charge value than to the ATP concentration.

MATERIALS AND METHODS

Organism—*Escherichia coli* K-12 strain PC 0294, purA⁻, thr⁻, leu⁻, proA⁻, gal⁻, strA⁻, mtl⁻, xyl⁻, was a gift from Dr. A. J. Clark. The metabolic block in the purine pathway is at the adenylosuccinate synthetase step.

Growth Conditions—Cells were grown aerobically in a standard...
growth medium containing 5 g of KH₂PO₄, 17 g of K₂HPO₄·3H₂O, 2 g of (NH₄)₂SO₄, 300 g of MgCl₂·6H₂O, 5 g of glucose, 200 mg of L-proline, 50 mg of L-threonine, 50 mg of L-leucine, 20 mg of adenine, and 0.2 mg of thiamine hydrochloride/liter. For glucose-limited growth, 300 mg of glucose were used per liter, and for adenine-limited growth, 7.5 mg of adenine were used per liter. The temperature for each experiment is indicated in the figure legends. Cells were checked for adenine requirement at the conclusion of each experiment to guard against contamination.

For the chemostat experiments, a cylindrical glass vessel 15.5 cm long and 11 cm in diameter, with integral baffles and fritted glass jacket, was used. The stopper was sealed with a rubber stopper. Holes were drilled in the stopper to accommodate the level controller probes, tubing for sampling, air input and exhaust, medium inflow and outflow, and antifoam addition. "Antifoam A" from Dow Corning Corp., a silicone emulsion in an aerosol spray can, was removed from the can and centrifuged at 10,000 × g for 15 min. Only the supernatant fraction was used. The volume of the culture in the chemostat was maintained at 500 ml by peristaltic pumps (one controlling inflow and one controlling outflow) functioning in conjunction with a Cole-Parmer Dyna-Sense level controller (model 7186). Aeration of the culture was achieved by passing moist air through a fritted glass sparger at a rate of 1 liter/min. A rapidly rotating Teflon-coated magnet was used for thorough mixing of the culture.

Perchloric Acid Extraction of Adenine Nucleotides—A 1-ml sample of the bacterial culture was removed through narrow tubing by suction and rapidly pipetted into 0.2 ml of 30% HClO₄. After 30 min at 0°, the extract was frozen at −70°. Within 24 hours, the extract was thawed, thoroughly mixed, and centrifuged at 12,000 × g for 3 to 4 min at about 4°. A sample of the supernatant fluid (0.5 ml) was neutralized with about 0.26 ml of a solution containing 2.6 M KOH and 0.58 M KHCO₃. After at least 15 min at 4°, the KClO₄ was removed by centrifugation. The samples were stored at −70° and assayed within 5 days of extraction. The ranges of recovered values for ATP, ADP, or AMP added to the perchloric acid at the time of cell addition and carried through the entire procedure were 95 to 100%, 101 to 103%, and 92 to 100%, respectively.

This extraction procedure has been modified from that previously used in this laboratory (9) by centrifugation of the perchloric acid extract. This removal of acid-prefracible material before neutralization of the extract improves the recovery of externally added adenine nucleotides, presumably by removal of enzymes that are otherwise reactivated on neutralization. With the modified procedure described here, the energy charge in exponentially growing E. coli was found to be about 0.9 rather than about 0.8 as previously reported. While this manuscript was in preparation, a paper appeared (24) recommending here, the energy charge in exponentially growing E. coli was found to be about 0.90. Within 1 hour after glucose was added to the chemostat culture, the energy charge decreased to about 0.90, and the total adenine nucleotide concentration decreased to about 20% of normal. These results are similar to those reported by Chapman et al. (9) for a wild type E. coli strain, except that the energy charge values are consistently higher by about 0.1 unit, a consequence of the improvement in the assay procedure noted above. Further experiments were designed to study the extent to which the energy charge is stabilized during large changes in the total adenine nucleotide concentration when an energy source is available.

RESULTS

Glucose Starvation—In a preliminary experiment, the adenine auxotroph E. coli PC 0294 was grown in medium containing a limiting amount of glucose. During exponential growth, the ATP level was about 10 nmol/mg of protein, which is similar (assuming that protein represents 50% of the cell's dry weight (28) to previously published values (29). The sum, ATP + ADP + AMP, was about 11 nmol/mg of protein. The energy charge was about 0.90. Within 1 hour after glucose was depleted, the energy charge had decreased to about 0.80, and the total adenine nucleotide concentration had decreased to less than 50% of normal. These results are similar to those reported by Chapman et al. (9) for a wild type E. coli strain, except that the energy charge values are consistently higher by about 0.1 unit, a consequence of the improvement in the assay procedure noted above. Further experiments were designed to study the extent to which the energy charge is stabilized during large changes in the total adenine nucleotide concentration when an energy source is available.

Adenine Starvation—E. coli PC 0294 was grown with a
limiting supply of adenine (the growth rate was the same as with excess adenine). At about the time that growth stopped, there was a dramatic drop in the adenylate pool (sum of ATP, ADP, AMP concentrations) to 10% or less of normal. These results are plotted in Fig. 1. Assuming that the protein concentration represents 50% of the cellular dry weight (28), and that there are 2.7 ml of intracellular water/g dry weight (30), we estimate that the intracellular adenine nucleotide concentration dropped from about 7 mM to 0.7 mM or less. The energy charge dropped from about 0.90 to about 0.70. Thus, a very large decrease in the adenylate pool size was associated with only a moderate drop in the energy charge. Growing the cells at 25°C instead of 37°C slowed the decline in the adenylate concentration so that the interim events could be observed more easily. The energy charge remained near the normal value of 0.9 while the adenylate pool level dropped to as low as 30% of normal (Fig. 2). As the adenylate level continued to decline, the energy charge dropped to about 0.7. In order to show these relationships more clearly, the energy charge has been plotted as a function of the adenylate concentration (Fig. 3). The cells continue to take up oxygen at a rate at least 70% of the control for 50 min after resuspension without adenine at 25°C (Fig. 4).

Fig. 1. Total adenine nucleotide concentration and energy charge in Escherichia coli PC 0294 grown on limiting adenine. Medium with limiting adenine (7.5 mg/liter), 0.1% glucose, and other supplements at standard concentrations was inoculated at time zero with cells from a late exponential or early stationary phase culture. The initial absorbance was about 0.04. The culture was incubated at 37°C and doubling time was 1.3 hours.

Fig. 2. Total adenine nucleotide concentration and energy charge in Escherichia coli PC 0294 grown on limiting adenine. Glucose medium with 7.5 mg of adenine and 17.5 mg of leucine/liter and other supplements at standard concentrations was inoculated at time zero with cells from a late exponential or early stationary phase culture. The initial absorbance was about 0.04. The culture was incubated at 37°C and doubling time was 1.3 hours.

Fig. 3. Energy charge as a function of the total adenine nucleotide concentration during adenine starvation. Data are replotted from Fig. 2. The initial total adenylate concentration of 11.6 nmol/mg of protein (average of the first five points in Fig. 2) was taken as 100%.

Fig. 4. Oxygen uptake by Escherichia coli PC 0294 during adenine starvation. Cells growing exponentially at 25°C in glucose minimal medium with standard supplement concentrations were collected by centrifugation at 4°C. Equal amounts of cells were resuspended in complete medium and in adenine-deficient medium. Three milliliters of each suspension (A 540 about 0.27) were incubated at 25°C with vigorous stirring. At intervals, the oxygen probe was placed in each chamber, preventing aeration, and the rate of oxygen uptake monitored for 5 min. The procedure was repeated, comparing cells resuspended in complete medium and glucose-deficient medium. The rate of oxygen uptake in each case was plotted as a percentage of the rate for cells in complete medium, which was 11% of the saturating oxygen concentration per min for a suspension with an absorbance at 540 nm of 1.0.

Adenine-limited Steady State Growth—When the adenine auxotroph was grown in adenine-limited chemostat cultures (Fig. 6), the adenine nucleotide concentration varied from 11.7 to 3.0 nmol/mg of protein as the dilution rate (fraction of chemostat volume replaced per hour) was decreased from 0.62 hour⁻¹ to 0.13 hour⁻¹, corresponding to an increase in doubling time from 1.1 to 5.3 hours. No simple relationship was noted between the decline in adenine nucleotide concentrations and the decline in the growth rate. The energy charge appeared to be constant, at an average value of 0.89 ± 0.01 (standard error of the mean) at all growth rates shown. The chemostat results...
thus confirm the conclusion reached from the batch culture experiments. Cells are able to maintain an energy charge value of about 0.90 while the adenine nucleotide level decreases to as low as 30% of normal.

**Protein Synthesis during Adenine Starvation or Glucose Starvation**—Since adenine starvation of an adenine auxotroph results in relatively independent variation of the ATP concentration and the energy charge, it is now possible to ask whether the rate of a biosynthetic process, such as protein synthesis, is correlated more closely with the ATP concentration or the energy charge. *E. coli* PC 0294 cells were grown with a limiting level of adenine, and the rate of protein synthesis was estimated as the amount of \[^{14}C\]leucine incorporated into hot trichloroacetic acid-insoluble material during a 1-min incubation. The results in Fig. 7 show that during adenine starvation the rate of \[^{14}C\]leucine incorporation remained constant for about 10 min after adenine was depleted, and then declined gradually over a 15-min period to about 25% of normal. This decline in protein synthesis began approximately as the ATP concentration dropped below 20% of normal and the energy charge dropped below 0.8. The rate of \[^{14}C\]leucine incorporation has been plotted as a function of the energy charge value or the ATP concentration in Fig. 8.

For a comparison with adenine limitation, *E. coli* PC 0294 cells were grown on a limiting amount of glucose. The rate of \[^{14}C\]leucine incorporation declined to less than 10% of normal during the first 10 min of glucose starvation (Fig. 9). The energy charge was 0.87 ± 0.01 (standard error of the mean) during the first 15 min of glucose starvation (the energy charge

---

**FIG. 5.** Effect of addition of adenine on the total adenine nucleotide concentration and energy charge in *Escherichia coli* PC 0294 resuspended in medium lacking adenine. Exponentially growing cells (doubling time 3.1 hours at 26°C) were collected by centrifugation (4') and resuspended at time zero in glucose medium containing normal supplements, except that the leucine concentration was 5 mg/liter and no adenine was present. The culture was incubated at 26°C. At 37 min, adenine was added to bring the concentration to 30 mg/liter.

**FIG. 6.** Adenine nucleotide concentrations and energy charge in adenine-limited steady state cultures of *Escherichia coli* PC 0294. The chemostat was described under "Materials and Methods." The inflow medium contained 7.5 μg of adenine/ml. Each point on the graph represents the mean of three samples taken at hourly intervals after the cell density had reached a steady state. Curve identifications: EC, adenylate energy charge; Σ, adenylate pool (sum of concentrations of ATP, ADP, and AMP).

**FIG. 7.** Rate of protein synthesis, adenine nucleotide concentration, and energy charge in *Escherichia coli* PC 0294 grown on limiting adenine-glucose medium, with 7.5 mg of adenine and 17.5 mg of leucine/liter and other supplements at standard concentration, was inoculated with exponential phase cells. The initial absorbance at 540 nm was 0.13. Growth stopped about 6 hours later at an A₅₄₀ of 0.63. Sampling began at an A₅₄₀ of about 0.58. The culture was incubated at 25°C and the doubling time was 2.5 hours. The rate of protein synthesis was expressed as nanomoles of \[^{14}C\]leucine incorporated per mg of protein during a 1-minute incubation. The amount of radioactivity incorporated was converted to amount of leucine incorporated as described in the appendix.

**FIG. 8.** Rate of protein synthesis as a function of the ATP concentration (A) or the energy charge (B) in adenine-limited cells. For each \[^{14}C\]leucine incorporation value, the ATP concentration or energy charge was read off the appropriate curve in Fig. 7. The average of the first five ATP values in Fig. 7, 10.6 nmol/mg of protein, was designated 100% in the upper figure (A).
during growth was 0.93 ± 0.02). The ATP concentration during this period was about 80% of the value during growth. In Fig. 10, the rate of [14C]leucine incorporation during the first 20 min of glucose starvation has been plotted as a function of the energy charge or ATP concentration. Comparison of these results with those shown in Fig. 8 indicates that protein synthesis is inhibited more rapidly and severely with regard both to time and to ATP concentration or energy charge.

When exponentially growing cells of E. coli PC 0294 were collected and suspended in medium lacking both adenine and glucose, the energy charge was about 0.80 (about 0.1 unit below the control culture), the total adenine nucleotide concentration was about 80% of control, and the rate of [14C]leucine incorporation was about 10% of control (Fig. 11). Functionally, the cells appear to be glucose limited and not as yet adenine limited, since the total adenine nucleotide concentration is stable at a relatively high level. After 20 min, glucose was added to the culture. Within about 1 min the energy charge recovered to the control value of about 0.90, the rate of [14C]leucine incorporation began to increase, and the total adenine nucleotide concentration began to decline, presumably because of nucleic acid synthesis. Although the ATP concentration was declining, the rate of [14C]leucine incorporation continued to increase, reaching almost the value observed in growing cultures, during the 5 min that the energy charge value remained above 0.90. As the energy charge dropped below 0.90, the rate of incorporation of [14C]leucine began to decline gradually, reaching about 40% of normal in about 10 min. The rate of protein synthesis is shown as a function of the energy charge and of the ATP concentration in Fig. 12.

**DISCUSSION**

For metabolite systems, such as the pyridine nucleotides or the adenine nucleotides, whose metabolic function is to interrelate sequences of reactions by serving as coupling agents between them, it is logical that ratios of concentrations, rather than absolute concentrations, would be the effective control parameters (31). Response to the mole fraction of the oxidized form (or the ratio of the oxidized to reduced form) of the pyridine nucleotides has been reported for dehydrogenases (32, 33) and response to the adenylate energy charge has been reported for many enzymes (1-8, 32, 33) studied in vitro. Enzymes that respond to variation in the energy charge (or in the pyridine nucleotide ratio) have been found to be insensitive to changes in the absolute concentration of the adenine nucleotides (or pyridine nucleotides) over considerable ranges around the presumed physiological levels (4, 7, 8, 32-34). These results support the predictions based on the logic of metabolic relationships.

A direct test of the importance of concentration ratios in the

![Fig. 9. Rate of protein synthesis, adenine nucleotide concentration, and energy charge in Escherichia coli PC 0294 grown on limiting glucose. Medium with 15 mg/liter of leucine, 0.0% glucose, and other supplements in the control culture was inoculated with late exponential phase cells. The initial absorbance at 540 nm was 0.15. Growth stopped about 6 hours later at an Amax of 0.96. Sampling began just as growth was stopping. Samples were also taken from a control culture containing the standard glucose concentration (0.5%). The cultures were incubated at 25°C, and the doubling time was 3.0 hours in both cultures. Protein synthesis was plotted as nanomoles of [14C]leucine incorporated per mg of protein during a 1-min pulse. The amount of radioactivity incorporated was converted to the amount of leucine incorporated as described in the appendix.](image)

![Fig. 10. Rate of protein synthesis as a function of ATP concentration (A) or energy charge (B) in glucose-limited cells. Data from the first 20 min of glucose starvation (Fig. 9) have been replotted. The average ATP concentration for five samples from the control culture, containing adequate glucose, 9.5 nmol/mg of protein, was taken as 100%.](image)

![Fig. 11. Rate of protein synthesis, adenine nucleotide concentration, and energy charge in Escherichia coli PC 0294 resuspended in medium lacking adenine and glucose. Exponentially growing cells (doubling time 2.7 hours at 27°C) were harvested by centrifugation (4°C) and resuspended at 27°C in medium containing standard supplement concentrations, except that the leucine concentration was 5 mg/liter and neither adenine nor glucose was present. For the control culture, adenine and glucose were present. Protein synthesis was plotted as nanomoles of [14C]leucine incorporated per mg of protein during a 1-min pulse. The amount of radioactivity incorporated was converted to the amount of leucine incorporated as described in the appendix.](image)
that a change of less than 10% in the energy charge affects over-all metabolism as reflected in growth more severely than charge in its normal range, macromolecular synthesis should grow when the energy charge has decreased by as much as 10% from its value in a rapidly growing culture. It thus seems possible to break the usual covariance of ATP concentration and energy charge. The energy charge rose rapidly because of its normal value except in the adenine auxotroph. Mechanisms for stabilizing the energy charge when the adenylate pool level is below 50% of normal will obviously not have evolved if the cell's homeostatic mechanisms maintain the pool level above 50%.

The effect of energy charge on growth is not, of course, exerted solely at the level of macromolecular synthesis. Rather, it must be the sum of effects on many processes, including the synthesis of the necessary building blocks, their activation (3), and their assembly into macromolecular products. When the energy charge fell in the adenine auxotroph as a result of extensive depletion of the adenylate pool, the rate of incorporation of labeled leucine into the protein continued nearly unabated while the charge decreased by about 15%, and then declined relatively slowly (Fig. 7). Presumably, the levels of intermediates fell during the period of declining protein synthesis. The level of messenger RNA also probably decreased, since either the lower energy charge or the low concentration of ATP as a substrate would be expected to prevent synthesis of RNA at a significant rate. In any case, although some protein synthesis occurred after the charge fell below the normal range, the results were compatible with the generalization that growth does not occur under these conditions, since leucine incorporation reached a very low level in much less than one generation.

When cells were starved for glucose rather than for adenine, the rate of incorporation of leucine into protein fell much more sharply than when adenine was limiting (Fig. 9). This difference presumably reflects the general deficiency of metabolic intermediates in the glucose-starved cells. Since the decrease in size of the adenylate pool is relatively small in these cells, the difference between incorporation as a function of ATP concentration and as a function of energy charge is much less pronounced in the glucose-starved cells (Fig. 10) than in those starved for adenine (Fig. 8).

When cells of the adenine auxotroph were suspended in medium lacking both glucose and adenine, the energy charge stabilized at a level below the normal growth range, and the constancy of the adenylate pool level indicates that there was no net synthesis of nucleic acid. By addition of glucose, it was possible to break the usual covariance of ATP concentration and energy charge. The energy charge rose rapidly because of the availability of a metabolic energy source. With the energy charge in its normal range, macromolecular synthesis should.

![Graph](image_url)
resume, and the expected decrease in the adenine nucleotide pool level is seen in Fig. 11. Protein synthesis also resumed, reaching nearly the rate seen in cells growing exponentially despite the rapid fall in the concentration of ATP. The rate of incorporation remained relatively high even after the concentration had fallen to about 10% of the level seen in growing cells, and its decline was approximately parallel with the decline in energy charge.

Since the first discussion of the adenylate energy charge as a metabolic control parameter, it has been emphasized that the effects of energy charge in vivo on any reaction must be modulated by other regulatory effects (10). The uniqueness of energy charge regulation lies in its ubiquity; virtually all metabolic sequences either utilize or regenerate ATP, and all may be expected to be regulated by the energy charge. But for any reaction or any sequence, energy charge is only one of the control parameters. Since the rate of the reaction or sequence is determined by interaction of many regulatory inputs, it cannot be predicted from knowledge of the extent of variation of any one of them. This situation is shown clearly in Fig. 11. When glucose was limiting, the energy charge was about 0.8 and there was virtually no incorporation of leucine. After addition of glucose, when the energy charge had fallen to 0.8 because of adenine deficiency (at about 30 min in the figure), the rate of leucine synthesis was over half as fast as in the growing culture. The difference between these two situations must be the much higher levels of all intermediates in the latter case.

Biological growth involves a more complex coordination of chemical reactions than any other known event or process. Clearly, very many concentrations and other parameters must be within acceptable limits for growth to occur, and the rate of growth at any given moment will be a function of the values of whichever parameters happen at that time to deviate from their optimal values. Further, the optimal value for any parameter may depend to some extent on the present values of others. It is to be expected that the acceptable ranges of some parameters will be relatively wide, and those of others will be narrow, depending on the metabolic functions of the compounds involved. Because the energy charge is one of the regulatory inputs to most, and probably all, metabolic sequences, even a slight decrease in its value must lead, through complex networks of primary, secondary, and more indirect effects, to changes in the rates of most sequences and in the concentrations of many intermediates. As a consequence of the amplification that must result from these metabolic cascades, it may be expected that the acceptable ranges of the ATP:ADP and ATP:AMP ratios and of the energy charge should be extremely narrow. That is, such an integrated process as growth should be strongly dependent on the value of the energy charge, and growth should cut off sharply as the energy charge declines past a narrow critical range. The results of the chemostat experiments (Fig. 6) are consistent with this expectation; growth was not observed at energy charge values detectably below normal. However, it is obvious that, when the energy charge is in the range compatible with growth, many other factors will affect both the ability of the cell to grow and the rate of growth. No net growth is possible, for example, in the absence of a nitrogen source, no matter how high the energy charge or the concentrations of intermediates of glycolysis and the citrate cycle. In the chemostat experiments reported here, the only ultimate limiting factor is the supply of adenine nucleotides. Thus, the situation is much simpler than in a glucose-limited culture, for example, where energy and nearly all metabolic intermediates will be in short supply. Even in the adenine-limited cultures, however, there will be secondary effects, and in view of the involvement of ATP in all metabolic sequences, they may be extensive. The decrease in growth rate observed in the chemostat experiment may have resulted rather directly from effects of lowered adenylate concentration on membrane function or from a decrease in the ability of the cell to synthesize nucleic acids, histidine, or nucleotide cofactors (which incorporate some of the atoms of ATP), or, less directly, from lowered concentrations of other intermediates or from a slightly lowered energy charge. A complex interaction of several of these effects seems most likely. In particular, it is impossible to determine from our results whether a slight decrease in energy charge accompanied, and perhaps participated in causing, the decrease in growth rate. There is no evidence, and no basis for conjecture, as to whether the value of the energy charge, once it was in the range allowing growth, was a rate-limiting factor.

Whatever the reasons for the decrease in growth rate as adenine became more severely limiting, the primary importance of the chemostat experiment lies in the observation that growth occurred over a 3-fold range of adenine nucleotide concentrations, but only when the energy charge was essentially constant. Thus, when the only primary limiting factor is the availability of adenine nucleotides, the intracellular concentrations of these compounds can be forced far below the normal range without preventing growth: but growth appears to be possible only when the adenylate concentration ratios, and the energy charge, are maintained within narrow normal limits.

REFERENCES

1. Atkinson, D. E. (1969) Annu. Rev. Microbiol. 23, 47-68
2. Bigler, W. N., and Atkinson, D. E. (1969) Biochem. Biophys. Res. Comm. 36, 381-386
3. Brenner, M., De Lorenzo, F., and Ames, B. N. (1970) J. Biol. Chem. 245, 450-452
4. Shen, L. C., and Atkinson, D. E. (1970) J. Biol. Chem. 245, 396-400
5. Liao, C. L., and Atkinson, D. E. (1971) J. Bacteriol. 106, 37-44
6. Miller, A., and Atkinson, D. E. (1972) Arch. Biochem. Biophys. 152, 531-538
7. Chaluvatnatol, M., and Atkinson, D. E. (1973) J. Biol. Chem. 248, 2712-2715
8. Sharp, P. D. (1975) FEBS Lett. 33, 348-350
9. Chapman, A. G., Fall, L., and Atkinson, D. E. (1971) J. Bacteriol. 106, 1092-1096
10. Atkinson, D. E. (1968) Biochemistry 7, 4030-4034
11. Lowry, O. H., Carter, J., Ward, J. B., and Glaser, L. (1971) J. Biol. Chem. 246, 6511-6521
12. Ball, W. J. (1973) Ph.D. thesis, University of California, Los Angeles
13. Barwell, C. J., and Hess, B. (1971) FEBS Lett. 19, 1-4
14. Harrison, D. E. F., and Maitra, P. K. (1969) Biochem. J. 112, 647-656
15. Dietzler, D. N., Lais, C. J., and Leckie, M. P. (1974) Arch. Biochem. Biophys. 160, 14-25
16. Miović, M. L., and Gibson, J. (1973) J. Bacteriol. 114, 86-95
17. Ushakov, V. D. (1971) J. Biol. Chem. 246, 1607-1617
18. Gurd, J. W., and Schoefield, P. G. (1971) Can. J. Biochem. 49, 686-694
19. Katchalski-Katzir, E., Kekomäki, M. P., and Mäenpää, P. H. (1969) Biochem. Pharmacol. 18, 2615-2624
20. Woods, H. F., Eggleston, L. V., and Krebs, H. A. (1970) Biochem. J. 119, 501-510
21. Slayman, C. L. (1973) J. Bacteriol. 114, 752-766
22. Woods, H. F., and Krebs, H. A. (1973) Biochem. J. 132, 55-60
23. Chagoya de Sánchez, V., Brunner, A., and Piña, E. (1973) Biochim. Biophys. Res. Commun. 48, 1441-1445
24. Davison, J. A., and Fynn, G. H. (1974) Anal. Biochem. 58, 632-637
25. Wilson, E. B., Jr. (1952) An Introduction to Scientific Research, pp.
26. Klungseeyr, L. (1969) *Anal. Biochem.* **27**, 91–98
27. Volkin, E., and Cohn, W. E. (1954) in *Methods of Biochemical Analysis* (Glick, D., ed) Vol. 1, pp. 287–305, Interscience Publishers, New York
28. Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T., and Britten, R. J. (1955) *Studies of Biosynthesis in Escherichia coli*, p. 28, Carnegie Institute of Washington, Publication No. 607
29. Holmes, W. H., Hamilton, I. D., and Robertson, A. G. (1972) *Arch. Mikrobiol.* **83**, 95–109
30. Winkler, H. H., and Wilson, T. H. (1966) *J. Biol. Chem.* **241**, 2200–2211
31. Atkinson, D. E. (1970) in *The Enzymes* (Boyer, P. D., ed) 3rd Ed, Vol. I, pp. 461–489, Academic Press, New York
32. Shen, L. C., and Atkinson, D. E. (1970) *J. Biol. Chem.* **245**, 2974–2979
33. Barnes, L. D., McGuire, J. J., and Atkinson, D. E. (1972) *Biochemistry* **11**, 4322–4328
34. Atkinson, D. E., and Walton, G. M. (1967) *J. Biol. Chem.* **242**, 3239–3241
35. Chapman, A. G., and Atkinson, D. E. (1973) *J. Biol. Chem.* **248**, 8309–8312
36. Schramm, V. L., and Leung, H. (1973) *J. Biol. Chem.* **248**, 8313–8315
Attachment for unknown paper: Conversion of protein synthesis in the presence of specific radiolabeled amino acids.

From the Department of Chemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90024

The rate of protein synthesis in the bacterial culture was measured by incubating a sample of the culture with labeled amino acids and monitoring the rate of radioactivity incorporation into the bacterial protein. In order to measure the rate of protein synthesis, labeled cells were grown in radioactive medium, and the rate of incorporation was determined by measuring the incorporation of radioactive amino acids into the bacterial protein. The cells were then harvested and assayed for the radioactivity content, which was used as a measure of the rate of protein synthesis.

The following equation was used to calculate the rate of protein synthesis:

\[ \frac{d\alpha}{dt} = \frac{d\text{radioactivity}}{dt} \times \frac{1}{\text{protein content}} \]

This equation expresses the rate of protein synthesis as the rate of change in radioactivity divided by the protein content. The rate of protein synthesis was then calculated as the slope of the linear portion of the rate of protein synthesis versus time curve.

Figure 1 shows the results of the experiment. The rate of protein synthesis is plotted as a function of time. The results indicate that the rate of protein synthesis increases with time, reaching a maximum at approximately 20 min after the start of the experiment.

REFERENCES

1. Smiles, D. S., and Dellos, D. R. (1955). Proc. Natl. Acad. Sci. USA.
Relation of growth and protein synthesis to the adenylate energy charge in an adenine-requiring mutant of Escherichia coli.
J S Swedes, R J Sedo and D E Atkinson

J. Biol. Chem. 1975, 250:6930-6938.

Access the most updated version of this article at http://www.jbc.org/content/250/17/6930

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/17/6930.full.html#ref-list-1