ENERGY CHARGE REGULATION IN PHOTOSYNTHETIC BACTERIA

Joseph W. Zilinsky*, Gary A. Sojka, and Howard Gest

Department of Microbiology, Indiana University
Bloomington, Indiana 47401

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Summary. The "energy charge" concept suggests that the relative concentrations of adenylate nucleotides in cellular pools define a signal system that is important in the regulation, and integration, of energy conversion and biosynthesis in growing cells. The results of experiments in which photosynthetic bacteria are subjected to treatments that could be expected to disturb the "normal" energy charge can be reasonably interpreted in terms of "energy charge control."

Atkinson and colleagues (1-3) have shown that the *in vitro* activities of certain enzymes involved in heterotrophic ATP regeneration are progressively inhibited as the Energy Charge (EC) [=(ATP + 0.5 ADP)/(ATP + ADP + AMP)] increases, especially at values greater than about 0.7, while activities of a number of ATP-utilizing biosynthetic enzymes are stimulated. This reciprocal response relationship suggests that EC is an important regulatory "quantity" in growing cells, and our previous observations (4-6) relating to integration of energy conversion and biosynthesis in the photosynthetic bacterium *Rhodopseudomonas capsulata* are consistent with this notion. In this communication, we describe new approaches for investigating the significance of EC in regulation of cell metabolism.

Methods. *Rps. capsulata* strain St. Louis (American Type Culture Collection No. 23782) was grown (34°) in a synthetic medium containing 0.4 percent DL-malate and 0.1 percent $(NH_4)_2SO_4$ as the carbon and nitrogen sources, res-

*Present address: Department of Biology, Wisconsin State University, Oshkosh, Wisconsin.

pectively (5). In some instances, the inorganic phosphate (P_i) content of the medium was decreased to less than 10 mM; to increase the buffer capacity of such media, 0.02 M tris(hydroxymethyl)aminomethane was added and the initial pH decreased to 6.5. For anaerobic growth, illumination was provided with Lumiline lamps. Such cultures were grown either in completely filled screw-cap tubes (selected to fit in a Klett-Summerson (KS) photometer), or in a device similar to that described by Willingham and Oppenheimer (7); with the latter arrangement, an argon atmosphere was present and argon pressure used to displace samples into a collecting tube. For dark aerobic growth (exogenous ATP experiments) the cells were grown in the same medium and the flask cultures agitated on a rotary shaker as specified. Growth rates were followed by making serial turbidity measurements using a KS photometer equipped with a No. 66 filter; the turbidity value up to at least 220 photometer units is proportional to bacterial mass (200 units is equivalent to 440 µg dry weight of cells per ml). Bacteriochlorophyll (BChl) concentration was determined *in situ* as described previously (8). Photophosphorylation activity was measured by the procedure given in Ref. 9.

Results and Discussion.

Effects of exogenous ATP. The BChl content of purple bacteria is an index of the quantity of energy-converting membrane in such organisms, and is inversely related to growth rate of the cells and/or light intensity (under photosynthetic conditions) (10). We have proposed (5) that EC is one of the actual signals governing synthesis of the BChl-membrane complex, in that high rates of ATP regeneration and correspondingly high EC values suppress activity of biosynthetic enzymes concerned (note that in contrast with "ordinary" biosynthetic enzymes, those involved in BChl-membrane synthesis may be in a special category since the "product" constitutes the ATP-*regeneration* machinery). Since EC, *in vivo*, is set by low concentrations of the adenylate nucleotides, introduction of relatively small quantities of exogenous ATP might be expected to artificially raise the EC significantly

and, thereby, to inhibit further production of BChl-membrane. Under conditions where continued BChl synthesis is required for cell multiplication, a growth inhibition should ensue (assuming that exogenous ATP cannot serve as an energy source for growth).

Cells grown in darkness under highly aerated conditions (50 ml of medium in a 2 liter Erlenmeyer flask, at 200 rpm) contain barely detectable quantities of BChl. When such cells are shifted to anaerobic conditions (in the anaerobic sampling device) and illuminated at low intensity (60 footcandles), BChl is preferentially synthesized for some time. In a typical experiment, the BChl contents at 5 and 20 hrs are 10 and 30 μg/mg dry weight of cells, respectively. Addition of 5 mM MgATP (equimolar $MgCl_2$ and ATP; filter sterilized) to the culture at the time of the shift results in levels of approximately 3 and 16 μg BChl/mg at corresponding times. Thus, the presence of equimolar Mg^{2+} and ATP causes a great reduction in the BChl content of the cells.

As noted in an earlier brief report (11), the suppressive effect of exogenous ATP on BChl synthesis results in a marked inhibition of growth when the energy source is photophosphorylation (i.e., anaerobically; Fig. 1). Under the conditions described, the inoculum cells must preferentially synthesize BChl before exponential growth is possible (12) and it would appear that MgATP strongly interferes with such synthesis. The "recovery" from the MgATP inhibition starting at about 26 hrs is apparently not due to destruction or disappearance of the bulk of the ATP added, since as much as 4.6 mM ATP remains detectable (by enzymatic analysis) in the medium.

Dark aerobic growth of Rps. capsulata, for which BChl is unnecessary, is virtually unaffected by MgATP (see Fig. 1). This result also constitutes a kind of control for the possibility that ATP affects photosynthetic growth by simply chelating an essential metal such as Mg^{2+}, which is necessary for BChl synthesis. Other control experiments showed that addition of 10 mM $MgCl_2$ or a "Mg^{2+} buffer" ($MgCl_2$ plus glycerol-1-phosphate; Ref. 13) did not reduce the effect of 5 mM ATP in suppressing BChl formation. Similarly, the possibility

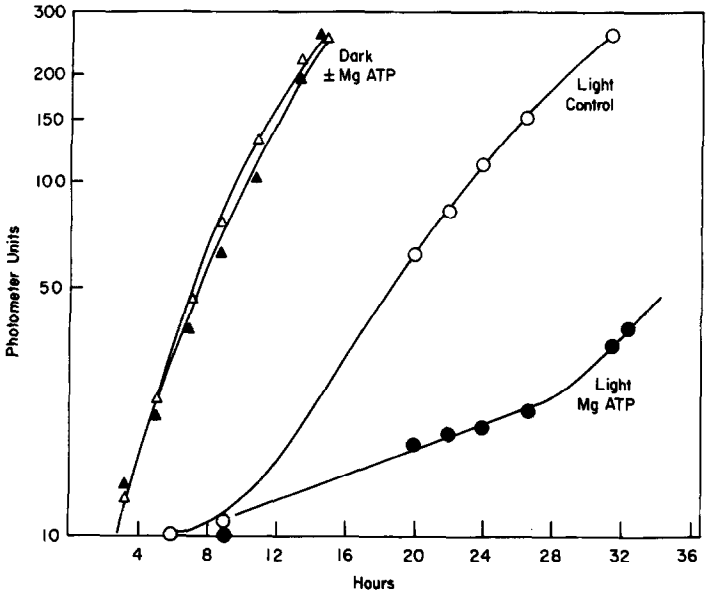

Figure 1. Effect of MgATP on growth of Rhodopseudomonas capsulata. Photosynthetic (light) cultures were grown in completely filled screw-cap tubes with 40 footcandles of illumination; dark cultures were grown with aeration in darkness, on a rotary shaker (50 ml of medium per 125 ml Erlenmeyer flask; 90 rpm). The BChl content of the inoculum cells, grown with vigorous aeration in darkness, was extremely low. Where indicated, 5 mM MgATP was added at zero time. △, aerobic dark control; ▲, aerobic dark plus MgATP; ○, light control; ●, light plus MgATP (note: at 43 hrs, this culture showed a density of approximately 150 photometer units).

that iron chelation might be involved is made unlikely by the observation that the effect of MgATP could not be alleviated by addition of ferric citrate to the medium. Thus, we believe it is likely that the effect is due to an influence of ATP as such, and thereby EC, at the sites of BChl-membrane synthesis.

Disturbance of EC by inorganic arsenate (As_i). As_i is a phosphorylation uncoupler and can substitute for P_i in numerous biochemical esterification reactions. Organic arsenate esters, however, are ordinarily extremely unstable in that they hydrolyze rapidly (14). Thus, the condensation product of ADP and As_i, produced by an energy conversion system, would be expected to have a very short half-life and, accordingly, is presumed to be unable to function as an energy source for biosynthesis or as a regulatory signal in place of

ATP. These considerations suggested that As_i might be useful as a probe for disturbing the adenylate nucleotide profile of growing cells.

Photosynthetic growth of Rps. capsulata was found to be sensitive to As_i, depending on the As_i/P_i ratio in the medium. With no As_i, and using 3 mM P_i, the mass doubling time (during logarithmic growth) in saturating light is of the order of 2 hrs. Addition of As_i has little effect until the As_i/P_i ratio exceeds about 0.2, at which point the doubling time begins to lengthen. At a ratio of 1, growth is severely inhibited, as shown in Fig. 2. Related experiments clearly showed that the absolute level of As_i (or P_i) is not the critical factor, but rather, the ratio As_i/P_i, i.e., the effect is competitive. It is evidently not easy to explain the magnitude of the growth inhibition with $As_i/P_i = 1$ as being due to a two-fold reduction in the rate of ATP regeneration, occasioned by the As_i versus P_i competition.

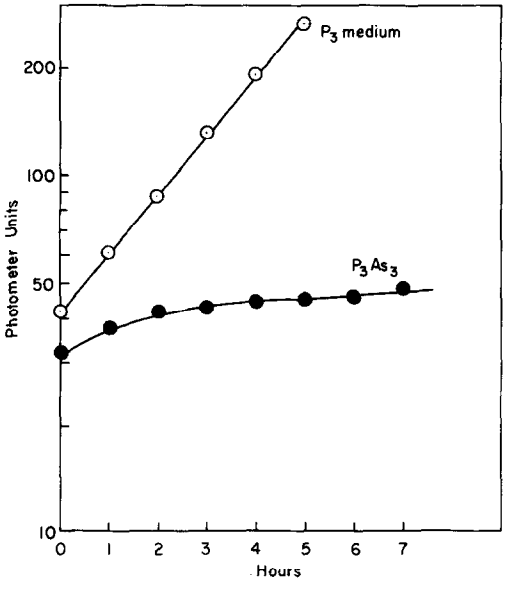

Figure 2. Effect of As_i on photosynthetic growth rate of wild type Rhodopseudomonas capsulata. P_3 refers to a medium containing 3 mM P_i; P_3As_3, 3 mM each of P_i and As_i. In both instances, the inoculum consisted of cells grown photosynthetically in P_3 medium. The inoculated experimental cultures, in completely filled screw-cap tubes, were incubated in saturating light (550 footcandles).

Arsenate-resistant mutants of Rps. capsulata were readily isolated as follows. In media containing 10 mM As_i and 10 mM P_i, cell multiplication

became evident after a long lag period. Serial transfers of such cultures were made, and cells from the fifteenth transfer were streaked on 0.3 percent yeast extract plus 0.3 percent peptone agar plates. The latter were incubated in darkness at 34°; red colonies were observed within 3 days and many of these proved to be due to As_i-resistant mutants. One such strain, designated as Z-1, has been examined in particular detail (15). The mass doubling time of Z-1 in 10 mM P_i medium (with saturating light) is approximately 100-110 min and its growth rate is not appreciably affected by the further addition of 10 mM As_i. An obvious explanation for As_i-resistance of Z-1 could be that the mutant is impermeable to As_i owing to a change in cytoplasmic membrane structure that allows discrimination between P_i and As_i. A series of experiments, however, showed no clear-cut difference between Z-1 and wild type cells in respect to As_i uptake; with both organisms, a very low net incorporation of $^{74}AsO_4^{3-}$ into the perchloric acid-insoluble cell residue was demonstrable. Additional information in this regard was obtained from experiments in which Z-1 and mutant cells were (separately) suspended in a "complete" medium containing a low concentration of As_i (6 µg/ml), but no P_i, and the suspensions illuminated; the slow rates of As_i disappearance from the medium were found to be essentially the same.

The possibility that the energy-converting system of Z-1 (in contrast to wild type) can discriminate between As_i and P_i at the subcellular level was tested by determining the photophosphorylation kinetics of membrane fragments. The experiments revealed that wild type and Z-1 particles have a similar Km for P_i (approximately 1 mM) and that photophosphorylation by both kinds of fragments is competitively inhibited by As_i, with about the same K_i. Significantly, however, the V_{max} (maximum velocity) of phosphorylation by Z-1 was found to be considerably higher; the average of 4 determinations was 280 µmoles P_i esterified/hr/mg BChl for Z-1, but only about 70 for wild type particles.

This difference in V_{max} suggests that the responses of wild type and Z-1 cells to As_i could be accounted for as follows. We assume that in the

presence of As_i, the *in vivo* rate of photophosphorylation may approach the V_{max}; rapid hydrolysis of ADP-arsenate should tend to keep the phosphorylation sites saturated with substrates, and in analogy with the effects of As_i on basically similar systems it is reasonable to believe that As_i accelerates light-dependent cyclic electron flow (the driving force of photophosphorylation). In wild type, decrease in the net rate of ATP regeneration due to the As_i versus P_i competition leads to substantial fall of EC to values dictating slow biosynthetic rates. Assuming further that the cells are unable to raise the EC to the optimal range, a severe growth inhibition would result. In Z-1, however, because of the markedly higher V_{max} of photophosphorylation, the cells are able to maintain the ATP regeneration rate and EC within the "normal" range despite the competition between As_i and P_i.

The interpretation advanced raises numerous questions subject to feasible experimental test. Indeed, one of the particularly valuable aspects of the EC regulation hypothesis is that it provides a new point of view for examining the dynamic interrelationships between energy conversion and biosynthesis.

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