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Growth, Luminescence, Respiration, and the ATP Pool During Autoinduction in *Beneckea harveyi*

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The bacterial bioluminescence system is unusual because it is self-induced. In the late logarithmic phase of growth, upon the accumulation of an autoinducer, the synthesis of the components of the system is initiated. We were interested in determining what effect this burst of synthesis and activity has on cellular energy metabolism. The ATP pool of the luminous bacterium *Beneckea harveyi* was found to dip 10- to 20-fold during the luminescence period, while the respiration per unit cell mass (optical density) increased but by much less. The dip in the ATP pool did not occur in four different types of dark mutants, including one that was temperature conditional and another that was conditional upon added cyclic AMP for luminescence. However, it is neither the synthesis nor the activity of luciferase that is responsible for the ATP dip; the dip does not occur in certain dark "aldehyde" mutants which nevertheless synthesize normal levels of luciferase, whereas it does occur at 36°C in a temperature-sensitive luciferase mutant which forms normal levels of inactive luciferase. Results with other aldehyde mutants implicate the pathway involved in the synthesis of the aldehyde factor with the ATP dip.

Bioluminescent bacteria in culture emit light at an appreciable rate, somewhere between 10^3 and 10^5 photons per s per cell, depending on the strain (11, 13). The enzyme responsible for the terminal light-emitting step is bacterial luciferase, which may be classed as a mixed-function oxidase in which reduced flavin mononucleotide and long-chain aliphatic aldehyde are oxidized by molecular oxygen.

$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{luciferase}} \text{light}$$

$$FMN + RCOOH + H_2O$$

The question as to what this light-emitting system represents in terms of energy expenditure has often been raised (10, 12). The energy expenditure is indeed substantial. Estimates based both on the cyanide-insensitive respiration (32) and the rate of photon emission compared to O_2 uptake (33) indicate that in fully induced cells (23) about 20% of the oxygen consumption of the cell goes via luciferase.

But these estimates take into consideration only the light emission itself. One must also consider the energy requirements for the biosynthesis of the luminescence system, which may also be substantial. Due to its unusual control, this synthesis can be distinguished experimentally from that of other systems. This is because the luminescent system is an induced one, postulated to involve an inducer, termed "autoinducer," produced by the cells themselves (8, 21, 23). The synthesis of luciferase and other components of the luminescent system occurs only after the autoinducer exceeds a certain level, after which time luminescence rises rapidly. In addition to luciferase itself, which ultimately constitutes at least 5% of the soluble protein of the cell (14), there are several other polypeptides, both soluble (18) and membrane-bound (24), which are coinduced with luciferase.

We considered that this induction period might represent a time when there is a major shift in the amount and/or pathway of energy expenditure, reflecting the new and specific synthesis. We therefore measured the ATP levels during this period and found a significant drop in the ATP pool (>10×), which appeared to be associated with the induction. Different mutants with different deficiencies associated with the luminescent system were examined in an attempt to understand the specific cause for the dip of intracellular ATP.

MATERIALS AND METHODS

Bacterial strains. *Beneckea harveyi* MAV, 392 (25) is designated as the wild type. Several mutants, including some temperature-conditional ones, were obtained after mutagenesis with nitrosoguanidine (4, 5): (i) TSL-1, temperature-sensitive luciferase; (ii)

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TSLS-1, temperature-sensitive luciferase synthesis; (iii) TSAS-1 and TSAS-F1, temperature-sensitive aldehyde synthesis (both are dim at the nonpermissive temperature but emit light when exogenous long-chain aldehyde is provided); (iv) cyclic AMP (cAMP)-requiring mutant, UY-437 (29); and (v) dark mutants M16, M17, and M18 (6), which also emit light with exogenous aldehyde. A dim mutant (AFM-1), which was obtained after treatment by acriflavin and fails to synthesize the luminescence system, was also used.

Media and conditions for growth. The rich medium designated as complex contains 0.3% yeast extract (Difco), 0.5% peptone (Difco), 0.01% $MgSO_4$, 3% NaCl, and 0.02 M morpholinopropane sulfonic acid (Sigma) buffer (pH 7.3). Minimal medium (pH 7.0) contains 3% NaCl, 0.7% $NaH_2PO_4 \cdot 7\ H_2O$, 0.1% K_2HPO_4, 0.01% $MgSO_4$, and 0.3% glycerol. Conditioned medium was prepared by growing wild-type cells to a density of about 5×10^8 per ml, then removing the cells by centrifugation and filtration and reautoclaving the medium (21). Growth was carried out in liquid cultures with shaking at temperatures specified between 27°C and 36°C. Cell density was determined in a Klett-Summerson photometer (filter number 66); a density of 100 Klett units is equal to 5×10^8 cells per ml growing in the complex medium and 1.2×10^9 cells per ml growing in the minimal medium.

Bioluminescence determination. In vivo bioluminescence of samples placed in a scintillation vial was measured in a photomultiplier photometer of Mitchell and Hastings (19), and expressed in quanta per second per milliliter using the standard of Hastings and Weber (15).

Oxygen uptake determination. Oxygen uptake was determined using a Clark-type oxygen electrode (Yellow Springs Instruments Co., Yellow Springs, Ohio). Three milliliters of a cell suspension containing no more than 5×10^7 cells per ml (higher densities were suitably diluted) was placed in the temperature-controlled oxygen cell. The rate of respiration was expressed in nanomoles of O_2 per minute per 10^6 cells.

ATP determination. Extraction and assay of the cellular ATP were based on the methods described by Addanki et al. (1) and Lundin and Thore (17), using a firefly lantern extract (Sigma FLE 250). The bacterial cultures (0.5 to 2.5 ml) were quickly harvested on a membrane filter (0.45 μm; Millipore Corp., Bedford, Mass.), which was then extracted for 5 min at 100°C in tris(hydroxymethyl)aminomethane (20 mM)-ethylenediaminetetraacetic acid (2 mM) buffer (pH 7.5), followed by cooling in an ice bath. Assays were performed in duplicate at room temperature by the addition of 0.4 ml of luciferase reagent to a 1-ml sample in a scintillation vial. In most experiments, counts were made for 1 s at 7 s after mixing, as described by Addanki et al. (1), in a liquid scintillation counter (Packard Tri-Carb, model 3314) on a tritium setting (gain 52%, discrimination, 50 to 100). In some experiments (Fig. 2, 4, 6, and 8), luminescence was measured with the photomultiplier photometer described above. The light intensity was recorded graphically during mixing, and the initial maximum intensity (at about 1 s) was taken as a measure of the ATP concentration. With both methods, the response was directly proportional to the ATP concentration. The ATP standard was freshly prepared and assayed at the time of the measurements. The standard deviation of 15 identical assays was ± 2.7%. The ATP content of the cells is expressed in picomoles per 10^6 cells.

RESULTS

Figure 1 shows measurements of growth, luminescence, respiration, and ATP pool of *B. harveyi* cells during growth in the complex liquid medium. During the initial stages of growth, prior to the develoment of luminescence, the cellular ATP pool and the respiration rate per 10^6 cells remained almost constant, at about 10 pmol of ATP and 0.3 nmol of O_2, respectively. At the time of the onset of light emission, the ATP pool fell by a factor of 10 or more within 60 min, while during this time the respiration rate per optical density unit increased by a factor of about 2 or, in some cases, less.

The dip in the ATP pool during the luminescence period was not due to a decrease in the oxygen tension, which occurs in the liquid culture medium during growth (22). Neither vigorous aeration nor shaking in an atmosphere of 100% oxygen gas prevented the dip. The specific composition of the complex medium also seemed not to be critical with regard to ATP level. Substitution for the morpholinopropane sulfonic acid buffer by 0.05 M sodium phosphate buffer, omitting the $MgSO_4$, or addition of 0.3% glycerol did not change the basic observation.

To find out whether the dip in the ATP pool is related to the synthesis or the activity of the luminescent system, we studied the ATP pool in three different mutants that fail to synthesize the luminescent system. In the dim mutant

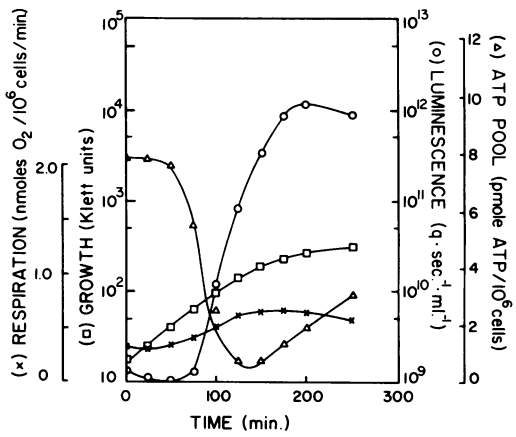

FIG. 1. *Growth, respiration, luminescence, and ATP pool in wild-type B. harveyi cells that were grown with shaking in complex medium at 27°C.* (□) *Growth;* (○) *luminescence;* (×) *respiration;* (△) *ATP pool.*

AFM-1, which emits only about 10^{-5} of the wild-type light, the ATP pool stayed almost constant throughout the growth cycle (Fig. 2), suggesting that the dip in the ATP pool is related to luminescence. Here again there was an increase in respiration during the time of induction.

The same result was found with two other dim mutants of *B. harveyi* whose lesions causing the failure to luminesce are known to be different and conditional. UY-437 is a cAMP-requiring mutant that does not emit light or synthesize luciferase in the absence of added cAMP (29). Figure 3 shows that if no cAMP is added this mutant does not exhibit a dip in its ATP pool during the expected time, whereas it does in the presence of added cAMP. TSLS-1 is a temperature-sensitive mutant which in its bioluminescence is like the wild-type at the lower (permissive) temperature, but at the higher (nonpermissive) temperature fails to synthesize luciferase (and other components of the luminescent system) (4, 24). With regard to the dip in ATP it was like the wild type at the permissive temperature, but, unlike the wild type, it showed a constant ATP during growth at the higher temperature (data not shown). Thus, three different dark mutants which fail to synthesize the luminescent system also failed to display the characteristic dip in the ATP pool.

From all these observations it would seem that the dip in the ATP pool is indeed associated with the luminescent system, and thus might also be triggered by the same factor which is believed to be responsible for the induction of the luminescent system (8, 23). Figure 4 shows that when log-phase *B. harveyi* cells were inoculated into a conditioned medium already containing inducer, the onset of the development of the luminescent system occurred immediately; similarly, the decrease in ATP in the cellular pool commenced without delay. At this new lower level (4.5 pmol of ATP per 10^6 cells), the ATP level stayed constant for about 2 h, after which, at the time of the onset of luciferase synthesis, a further dip occurred. For comparison, this figure also shows the ATP pool during growth of *B. harveyi* cells in fresh medium; here also the drop in the ATP pool was correlated with the onset of luciferase synthesis.

Two cellular components of the bacterial luminescent system known to be synthesized during the rise in light emission are the enzyme luciferase and an aldehyde-like factor. To find out whether it is the activity of the luciferase system that is responsible for the fall of the ATP pool, we utilized a mutant (TSL-1) that synthesizes all components of the luminescence system but is nonluminescent at 36°C because the luciferase is temperature sensitive (4). Since a dip in the ATP pool also occurred in TSL-1 at 36°C (Fig. 5), the decreased ATP is not due to luciferase activity, unless perhaps electrons pass unabated via a dark reaction of denatured luciferase. The dip in TSL-1 was similar to that exhibited by the wild type growing under the same conditions, but at 36°C the magnitude and duration of the dip was not as great as it was at 27°C in both strains.

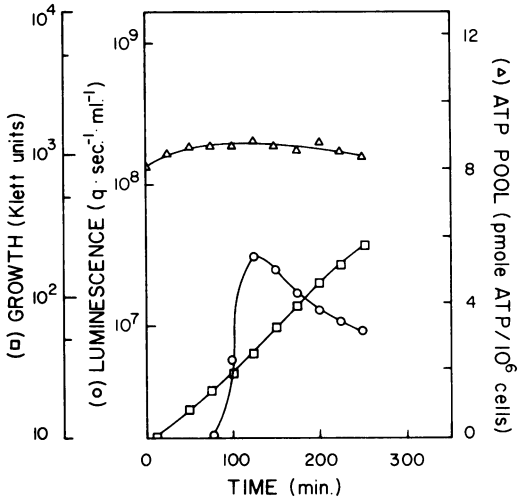

FIG. 2. *Growth, respiration, luminescence, and ATP pool in the dim mutant AFM-1. AFM-1 cells were grown with shaking in complex medium at 27°C.* (□) *Growth;* (○) *luminescence;* (△) *ATP pool.*

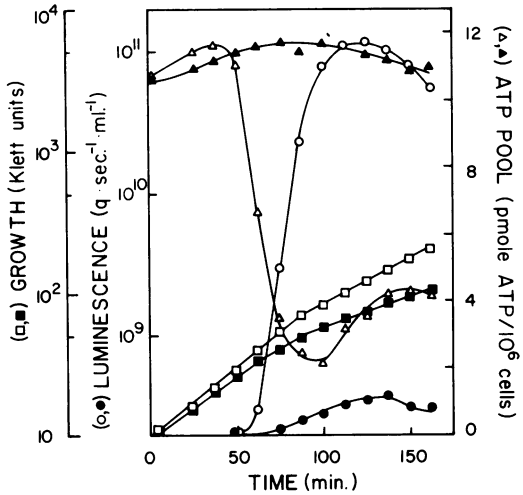

FIG. 3. *Growth, luminescence, and ATP pool in the cAMP-requiring mutant UY-437 in the presence and in the absence of cAMP. Cells were grown with shaking in complex medium at 28°C in the presence (open symbols) or absence (filled symbols) of 100 μg of cAMP per ml.* (□) *Growth;* (●, ○) *luminescence;* (▲, △) *ATP pool.*

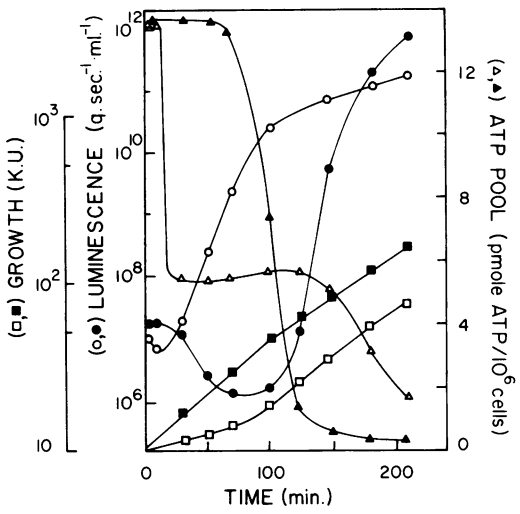

FIG. 4. *Effect of conditioned medium on ATP pool of wild-type cells. Cells were grown at 27°C in sterile complex media without (filled symbols) and with (open symbols) conditioning, achieved before the experiment by growth of cells to a density of 3 × 10⁸ cells. (■, □) growth; (●, ○) luminescence; (▲, △) ATP pool.*

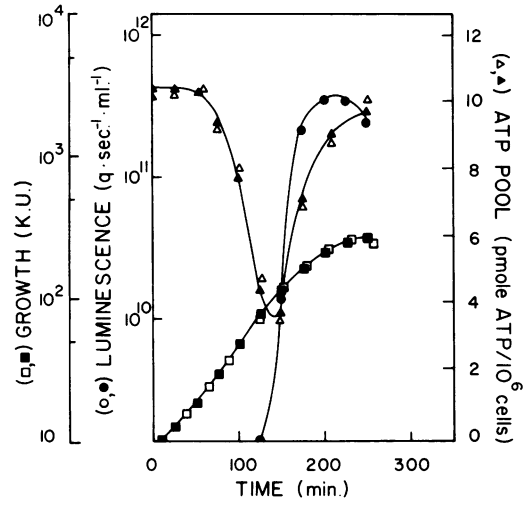

FIG. 5. *ATP pool in the mutant TSL-1 and wild-type strain MAV cells at 36°C. Wild-type (filled symbols) and TSL-1 (open symbols) cells were grown separately in complex medium at 36°C with shaking. (■, □) growth; (●, ○) luminescence; (▲, △) ATP pool. TSL-1 cells did not emit detectable light under these conditions.*

Could the dip in ATP be due to the synthesis of the luciferase? It appears not. The aldehyde mutants (M16, M17, M18) synthesize normal levels of luciferase but not the aldehyde factor and may thus be stimulated to emit light in vivo by exposing cells to long-chain fatty aldehydes. Figure 6 shows that none of this group of mutants showed a dip in the ATP pool during the growth cycle, thus excluding luciferase synthesis while implicating aldehyde biosynthesis or activity in the phenomenon. This suggestion is confounded by certain other aldehyde mutants, TSAS-1 and TSAS-F1 (6, 13), in which a typical dip in the ATP pool occurred not only at 25°C (data not shown) but also at the nonpermissive temperature (Fig. 7). These two temperature-sensitive mutants failed to luminesce at the nonpermissive temperature (36°C), but normal luminescence could be restored by added aldehyde (see Fig. 7). They differ from one another by the fact that, upon transfer from permissive to nonpermissive conditions, luminescence is rapidly lost in TSAS-F1, whereas in TSAS-1 the loss of luminescence requires a much longer time (6).

It has recently been found in our laboratory (28) that, although all of these dim mutants give bioluminescence with exogenously added aldehyde, myristic acid will also stimulate luminescence in the first class (M16, M17, M18). We therefore postulated that a long-chain acid is a precursor of aldehyde and that the two types of aldehyde mutants differ with regard to the location of the lesion in the aldehyde synthesis pathway, the "M" mutants being blocked prior to and the TSAS class subsequent to fatty acid formation. Since the M mutants would then lack the specific endogenous fatty acid, it was of interest to see whether added myristic acid would also cause a dip in ATP (Fig. 8). Within 25 min after the addition of myristic acid, the cellular pool of ATP fell by a factor of 10, and at the same time the respiration rate per cell was increased by more than twofold.

All of the above studies were carried out using a complex medium for growth of the cells. However, *B. harveyi* cells grown in a minimal medium may be very dim; less luciferase and other components of the luminescent system are synthesized (7, 23). Under these conditions, no dip in the ATP pool occurred (Fig. 9). However, arginine added exogenously will specifically stimulate the synthesis of the luminescence system in cells growing in a minimal medium (7, 23). Under these conditions also, no dip in the ATP pool was observed (Fig. 9). The explanation for this is not evident, though it may be related to the fact that the ATP pool of cells growing in minimal medium with or without arginine is at a considerably lower level (~3 pmol of ATP per 10^6 cells) than is found in cells growing in a rich medium. Another relevant point is that exogenously added myristic acid (20 μM) is much less active on cells growing in the minimal medium.

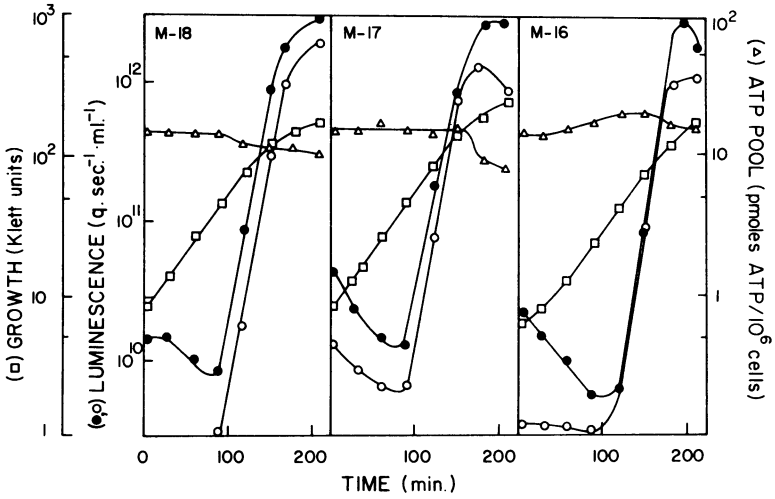

FIG. 6. *ATP pool in aldehyde dark mutants of B. harveyi. Dark-mutant M16, M17, and M18 cells were grown with shaking in complex medium at 27°C. (□) growth; (△) ATP pool; (●, ○) luminescence (filled symbols, with 5 μM decyl aldehyde; open symbols, without added aldehyde). Values for luminescence in the absence of aldehyde are multiplied by various factors: 10^2 for M16, 10^4 for M17, and 10^3 for M18.*

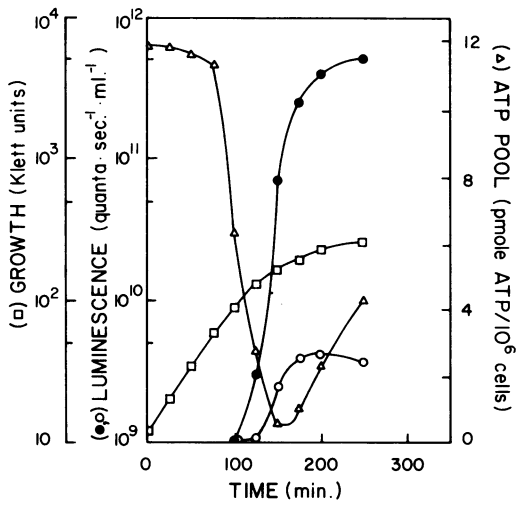

FIG. 7. *ATP pool in aldehyde dark mutant TSAS-1 at 36°C. Mutant TSAS-1 cells were grown with shaking in complex medium at 36°C. (□) growth; (●, ○) luminescence in the presence (filled symbols) or absence (open symbols) of 5 μM decyl aldehyde added to a sample assayed at the times noted; (△) ATP pool.*

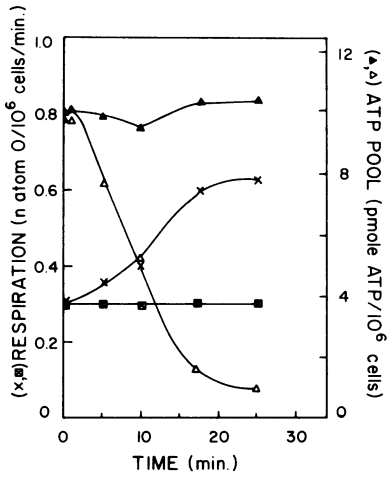

FIG. 8. *Effect of myristic acid on the ATP pool and respiration rate of M17 cells. M17 cells (50 ml) were placed in two flasks that were incubated at 27°C with shaking. To one flask myristic acid in ethanolic solution was added twice (at zero time and after 7.5 min) to a final concentration of 10 μM. To the second flask ethanol was added at the same concentration (0.001%) as a control. ATP pool (△) with and (▲) without myristic acid; respiration rate (×) with and (⊠) without myristic acid, respectively. Growth was not significantly affected over this time; viable cell counts were 2.2 × 10^8 cells per ml in both flasks at the end of the experiment.*

DISCUSSION

The drastic fall in ATP that occurs during the first stages of *B. harveyi* bioluminescence seems to be somehow linked to the light-emitting system. In four distinctly different types of dark mutants, two of them temperature conditional, the ATP dip did not occur. These include some of the aldehyde dark mutants (the M class) and three other types of dark mutants which fail to synthesize luciferase and other components of the luciferase system: AFM-1; TSLS-1, in which the synthesis of the luminescent system is con-

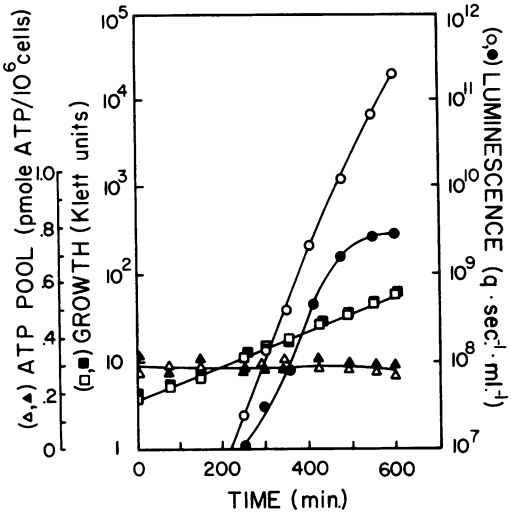

FIG. 9. *Growth, luminescence, and ATP pool in B. harveyi cells grown in minimal medium. Cells were grown at 27°C with shaking in the presence (6 × 10^{-3} M; filled symbols) and absence (open symbols) of* L-*arginine.* (■, □) *Growth;* (●, ○) *luminescence;* (▲, △) *ATP pool.*

ditional with temperature; and UY-437, in which the synthesis is dependent upon exogenously added cAMP. But it seems clear that it is neither the synthesis of luciferase nor its luminescence activity that is responsible. This was deduced by the facts that (i) the dip did not occur in the M class of dark aldehyde mutants which nevertheless synthesize normal levels of luciferase, while (ii) it did occur at 36°C in a temperature-sensitive luciferase mutant which synthesizes normal levels of inactive luciferase.

The different results with different dark aldehyde mutants implicate the pathway involved in the synthesis or activity of the aldehyde factor in the dip in ATP. In the TSAS mutants there is a dip in the ATP, whereas in the M mutants there is not. Assuming that this difference can be attributed to the locations of the lesions, it might be that of the ATP dip is due to the endogenous fatty acid, which would be formed in the TSAS class and not in the M mutants.

One explanation for the way in which the postulated endogenous long-chain fatty acid could cause a dip in the ATP pool involves the utilization of reducing power in its reduction to aldehyde. Failure to luminesce in TSAS would be due to some block subsequent to the formation of aldehyde, which itself would then be oxidized directly by oxygen without light emission. This explanation is difficult to accept because TSAS gives luminescence with exogenous aldehyde. However, there is evidence that, in addition to myristic acid, some other lipid factor is required for luminescence in vivo. The antibiotic cerulenin, which blocks fatty acid synthesis as its primary action (30), inhibits the development in vivo of myristic acid-stimulated luminescence in mutant M17, while not altering the response to exogenous aldehyde (27). TSAS mutants, which do not give bioluminescence with exogenous myristic acid, might be blocked at some step involved in the reaction of endogenous (but not exogenous) aldehyde with luciferase. The cycling of the endogenous fatty acid might thus continue unabated in the TSAS mutants.

An alternative explanation for the ATP dip is that endogenous fatty acid acts to uncouple oxidative phosphorylation. In this interpretation, TSAS accumulates fatty acid, whereas it is not formed in the M mutants. Long-chain fatty acids are known as uncouplers in both mitochondrial (3, 31) and bacterial systems (9). The mode of action and type of inhibition produced depend on several factors, including concentration (9, 26). Both exogenous and endogenous (16) fatty acids have been shown to activate the latent dinitrophenol-induced adenosine triphosphatase activity and to uncouple oxidative phosphorylation. The mechanism involved is generally believed to be associated with membrane effects. If, as proposed by Mitchell (20), the generation of chemical energy in the cell involves transmembrane potentials, it is possible that fatty acids uncouple the system by disrupting the membrane.

In fact, neither of these explanations seems very attractive. Another possibility for the dip in ATP comes from experiments of D. Karl and K. H. Nealson (personal communication). They confirmed the dip in ATP, but discovered that this decrease in accompanied by an increase in GTP. It may be that the fatty acid or some other intermediate is involved in a stimulation of a kinase catalyzing phosphate transfer from ATP to form GTP. These findings are not easy to reconcile with the idea that a shift in the electron flow is responsible for the ATP dip.

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ADDENDUM IN PROOF

In further experiments D. Karl and K. Nealson found a heat-stable adenosine triphosphatase activity in crude extracts which, because it increases in activity about the time of the ATP dip, might be responsible for some of the observations. However, it is still not

clear why the dip does not occur in dim and dark mutants, nor is it evident why there is a recovery of the intracellular ATP level subsequent to the dip.

LITERATURE CITED

1. **Addanki, S., J. F. Sotos, and P. D. Rearick.** 1966. Rapid determination of picomole quantities of ATP with a liquid scintillation counter. Anal. Biochem. **14**:261–264.
2. **Borst, P., and J. A. Loos.** 1959. The identification of the active component of mitochrome, and the influence of long-chain fatty acids on the adenosine triphosphatase activity of rat-liver mitochondria. Recl. Trav. Chim. Pays Bas **78**:874–875.
3. **Borst, P., J. A. Loos, E. J. Christ, and E. C. Slater.** 1962. Uncoupling activity of long-chain fatty acids. Biochim. Biophys. Acta **62**:509–518.
4. **Cline, T. W., and J. W. Hastings.** 1971. Temperature-sensitive mutants in the bacterial bioluminescence system. Proc. Natl. Acad. Sci. U.S.A. **68**:500–504.
5. **Cline, T. W., and J. W. Hastings.** 1972. Mutationally altered bacterial luciferase. Implications for subunit functions. Biochemistry **11**:3359–3370.
6. **Cline, T. W., and J. W. Hastings.** 1974. Bacterial bioluminescence in vivo: control and synthesis of aldehyde factor in temperature-conditional luminescence mutants. J. Bacteriol. **118**:1059–1066.
7. **Coffey, J. J.** 1967. Inducible synthesis of bacterial luciferase: specificity and kinetics of induction. J. Bacteriol. **94**:1638–1647.
8. **Eberhard, A.** 1972. Inhibition and activation of bacterial luciferase synthesis. J. Bacteriol. **109**:1101–1105.
9. **Galbraith, H., and T. B. Miller.** 1973. Effect of long chain fatty acids on bacterial respiration and amino acids uptake. J. Appl. Bacteriol. **36**:659–675.
10. **Harvey, E. N.** 1952. Bioluminescence. Academic Press Inc., New York.
11. **Hastings, J. W.** 1968. Bioluminescence. Annu. Rev. Biochem. **37**:597–630.
12. **Hastings, J. W.** 1975. Bioluminescence: from chemical bonds to photons, p. 125–146. *In* G. E. W. Wolstenholme and D. W. Fitzsimons (ed.), Energy transformation in biological system, Ciba Foundation Symposium 31. Associated Scientific Publishers, Amsterdam.
13. **Hastings, J. W., and K. H. Nealson.** 1977. Bacterial bioluminescence. Annu. Rev. Microbiol. **31**:549–595.
14. **Hastings, J. W., W. H. Riley, and J. Massa.** 1965. The purification, properties and chemiluminescent quantum yield of bacterial luciferase. J. Biol. Chem. **240**:1473–1481.
15. **Hastings, J. W., and G. Weber.** 1963. Total quantum flux of isotropic sources. J. Opt. Soc. Am. **53**:1410–1415.
16. **Hülsmann, W. C., W. B. Elliott, and E. C. Slater.** 1960. The nature and mechanism of action of uncoupling agents present in mitochrome preparations. Biochem. Biophys. Acta **39**:267–276.
17. **Lundin, A., and A. Thore.** 1975. Analytical information obtainable by evaluation of the time course of firefly bioluminescence in the assay of ATP. Anal. Biochem. **66**:47–63.
18. **Michaliszyn, G. A., and E. A. Meighen.** 1976. Induced polypeptide synthesis during the development of bacterial bioluminescence. J. Biol. Chem. **251**:2541–2549.
19. **Mitchell, G., and J. W. Hastings.** 1971. A stable inexpensive solid state photomultiplier photometer. Anal. Biochem. **39**:243–250.
20. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41**:445.
21. **Nealson, K. H.** 1977. Autoinduction of bacterial luciferase: occurrence, mechanism and significance. Arch. Microbiol. **112**:73–79.
22. **Nealson, K. H., and J. W. Hastings.** 1977. Low oxygen is optimal for luciferase synthesis in some bacteria: ecological implications. Arch. Microbiol. **112**:9–16.
23. **Nealson, K. H., T. Platt, and J. W. Hastings.** 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**:313–322.
24. **Ne'eman, Z., S. Ulitzur, D. Branton, and J. W. Hastings.** 1977. Membrane polypeptides co-induced with the bacterial bioluminescent system. J. Biol. Chem. **252**:5150–5154.
25. **Reichelt, J. L., and P. Baumann.** 1973. Taxonomy of the marine, luminous bacteria. Arch. Mikrobiol. **94**:283–330.
26. **Scholefield, P. G.** 1963. Fatty acids and their analogues, p. 153–172. *In* R. M. Hochster and J. H. Quastel (ed.), Metabolic inhibitors, vol. 1. Academic Press Inc., New York.
27. **Ulitzur, S., and I. Goldberg.** 1977. Sensitive, rapid, and specific bioassay for the determination of antilipogenic compounds. Antimicrob. Agents Chemother. **12**:308–313.
28. **Ulitzur, S., and J. W. Hastings.** 1978. Myristic acid stimulation of bacterial bioluminescence in aldehyde mutants. Proc. Natl. Acad. Sci. U.S.A. **75**:266–269
29. **Ulitzur, S., and J. Yashphe.** 1975. An adenosine 3′,5′-monophosphate-requiring mutant of the luminous bacteria *Beneckea harveyi*. Biochim. Biophys. Acta **404**:321–328.
30. **Vance, D., I. Goldberg, O. Mitsuhashi, K. Bloch, S. Omura, and S. Nomura.** 1972. Inhibition of fatty acid synthetases by the antibiotic cerulenin. Biochem. Biophys. Res. Commun. **48**:649–656.
31. **Van den Bergh, S. A., C. P. Modder, J. H. M. Souverijn, and H. C. J. M. Pierrot.** 1969. Some new aspects of fatty acid oxidation by isolated mitochondria, p. 137–144. *In* L. Ernster and Z. Drahota (ed.), Mitochondria structure and function, vol. 17. Academic Press Inc., London.
32. **VanSchouwenburg, K. L., and J. G. Eymers.** 1936. Quantum relationship of the light-emitting process of luminous bacteria. Nature (London) **138**:245.
33. **Watanabe, T., N. Mimura, A. Takimoto, and T. Nakamura.** 1975. Luminescence and respiratory activities of *Photobacterium phosphoreum*. J. Biochem. **77**:1147–1155.