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Chemomechanical Coupling without ATP: The Source of Energy for Motility and Chemotaxis in Bacteria

(oxidative phosphorylation/electron transport/flagella)

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ABSTRACT **The source of energy for bacterial motility is the intermediate in oxidative phosphorylation, not ATP directly. For chemotaxis, however, there is an additional requirement, presumably ATP. These conclusions are based on the following findings. (*i*) Unlike their parents, mutants of *Escherichia coli* and *Salmonella typhimurium* that are blocked in the conversion of ATP to the intermediate of oxidative phosphorylation failed to swim anaerobically, even when they produced ATP. When respiration was restored to the mutants, motility was simultaneously restored. (*ii*) Carbonylcyanide *m*-chlorophenylhydrazone, which uncouples oxidative phosphorylation, completely inhibited motility even though ATP remained present. (*iii*) Arsenate did not inhibit motility in the presence of an oxidizable substrate, though it did reduce ATP levels to less than 0.3%. (*iv*) Arsenate completely inhibited chemotaxis under conditions where motility was normal.**

In eukaryotes the transduction of chemical into mechanical energy (movement) involves ATP whenever it has been determined. Thus muscles contract and cilia and flagella beat by a sliding filament mechanism that uses ATP directly (1–3). In prokaryotes the immediate source of energy used in chemomechanical coupling is unknown.

Cells can generate ATP either by oxidative phosphorylation, resulting from the oxidation of substrates via electron transport, or by substrate-level phosphorylation, even anaerobically as in glycolysis. The intermediate form¶ of energy for oxidative phosphorylation has not yet been identified. This intermediate can be generated in bacteria either by electron transport or "backwards" from ATP by means of a Mg^{++},Ca^{++}-dependent adenosinetriphosphatase (Mg^{++},-Ca^{++}-ATPase) (4–12 and refs. 2, 5, 30–33 in ref. 10). See Fig. 4.

For certain bacterial functions that require energy, ATP is used directly; while for other functions, such as the energy-dependent transhydrogenase (11 and refs. 30–33 in ref. 10) and the transport of some amino acids (7–9, 12) and sugars (5, 10 and refs. 2 and 5 in ref. 10), the energy source is this intermediate of oxidative phosphorylation, without ATP being required.

The question raised in this paper is: What is the source of energy for motility and chemotaxis, ATP or the intermediate in oxidative phosphorylation? Several previously reported results do not answer this question since equilibration between ATP and the intermediate of oxidative phosphorylation was not prevented: motility and chemotaxis of a *Pseudomonas* strain (13) and of *Escherichia coli* K12 (14, 15) occurred anaerobically as well as aerobically, and the motility of *Pseudomonas fluorescens* was inhibited by uncouplers and respiratory inhibitors (16). Fields and Luria found that colicin E1 stops the motility of *E. coli*; ATP was reduced about one-third, but a known ATP-dependent reaction no longer occurred (17, 18). See the excellent review of this problem by Harold (pp. 214–217 of ref. 6).

Using mutants of *E. coli* and *Salmonella typhimurium* that are blocked at various stages of oxidative phophorylation, as well as employing certain inhibitors, we now show that the immediate source of energy for motility is not ATP but rather some intermediate in oxidative phosphorylation. Chemotaxis, however, requires an additional factor, presumably ATP.

MATERIALS AND METHODS

Chemicals and Media. From P. G. Heytler, via H. A. Lardy, we obtained carbonylcyanide *m*-chlorophenylhydrazone (CCCP), which is now available from Sigma Chemical Co., St. Louis, Mo. Minimal medium and tryptone broth were prepared as described previously (19). Chemotaxis medium consisted of 0.1 mM ethylenediaminetetraacetic acid and 10 mM potassium phosphate buffer, pH 7.0. Arsenate medium contained 0.1 mM ethylenediaminetetraacetic acid and 10 mM potassium arsenate, pH 7.0.

Bacteria. The following strains defective in oxidative phosphorylation were used: (*i*) *unc*A (*unc*oupled), AN120, an *E. coli* K12 mutant shown to be lacking oxidative phosphorylation and the Mg^{++},Ca^{++}-ATPase (4); (*ii*) ATPase-negative RV5 (isolated by R. C. Valentine), another *E. coli* K12 mutant missing this ATPase and, therefore, presumably lacking oxidative phosphorylation [similar to DL-54 (8), personal communications from R. D. Simoni]; (*iii*) *etc* (electron transfer coupling), TH31, a *S. typhimurium* mutant having this ATPase and phenotypically similar to *unc*A mutants of *E. coli* but defective in respiration-linked active transport (9). The isogenic parents of these mutants are AN180 (4), 1100, and TH32 (9), respectively.

Other *E. coli* K12 strains wild-type for oxidative phosphorylation, motility, and chemotaxis were: B14 (20), 20SOK$^-$ (20), and AW574 [a *gal*$^+$ *su*$^-$ derivative of AW405 (19)].

Abbreviations: Mg^{++},Ca^{++}-ATPase, Mg^{++},Ca^{++}-dependent adenosinetriphosphatase; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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¶ The nature of this intermediate form could be high energy compounds, an energized configurational state, a proton gradient [for a review see Harold (6)].

TABLE 1. *The effect of different energy sources on motility under anaerobic conditions*

Energy source added†	Time required to stop swimming*	
	*unc*A, ATPase, *etc*	All three parents
D-Lactace or succinate	10 min	10 min
D-Glucose or D-galactose‡	10 min	>24 hr

Cells grown in D-galactose minimal medium or tryptone broth gave similar results.

* Based on microscopic observations, loss of motility presumably resulted from using up the oxygen, since motility was immediately restored to all cells by removing the coverslip.

† With no energy source added, the bacteria slowed down gradually over a period of hours, presumably because the low rate of oxygen utilization led to anoxia slowly.

‡ The *unc*A mutant and its parent are *gal*$^-$; hence results for galactose do not apply for these strains; in the presence of galactose they behaved as if no energy source were added.

Bacteria were grown on tryptone broth unless stated otherwise. Only cells grown on glycerol minimal medium or tryptone broth were used in experiments with arsenate since these media, but not D-galactose minimal medium, produced cells sensitive to arsenate. This is in agreement with, and an extension of, results of Malamy and Bennett (21) who showed that glycerol, but not D-glucose, induces a phosphate transport system that transports arsenate.

The bacteria were washed free of growth medium by centrifugation three times in chemotaxis medium or, if specified, arsenate medium at room temperature, and then suspended in the respective medium.

Chemotaxis and Motility Assays. The *aerobic* assays have been described in detail previously (19). Briefly, the number of bacteria that swim into a capillary tube containing (*chemotaxis assay*) or not containing (*motility assay*) an attractant was determined. For the *anaerobic motility assay*, the cell reservoir was constructed of a stopcock grease well (Lubriseal) on a microscope slide, the cell suspension was added, and a second glass slide [or cover slip for microscopic observations (14)] was placed on top to seal the chamber. At 20 min, after the oxygen was used up (14), capillaries (sealed end first) were drawn completely through one grease wall and half way through the opposite wall such that the mouth of the capillary rested in the center of the cell suspension. The number of bacteria in the capillary was determined. For aerobic assays the bacterial concentration was 2×10^7 cells per ml, and for anaerobic assays, to hasten the depletion of oxygen, 10^9 cells per ml. In each of the above chemotaxis and motility assays, averages were reported for duplicate or quadruplicate determinations. Dark field microphotography was performed according to Dryl (22) and Doetsch (23).

ATP Assay. ATP was extracted (7) from cells washed in chemotaxis medium or arsenate medium and assayed by the luciferin–luciferase method (24).

Measurement of D-Glucose Metabolism. The details of this measurement are given in the Ph.D. thesis (in preparation) of S. H. Larsen. By use of D-[*U*-^{14}C]glucose, D-glucose was determined by scanning paper chromatograms, CO_2 as radioactivity lost upon acidification, and phosphorylated sugars by failure to stick to a Dowex-1 column after treatment of the anionic material with alkaline phosphatase.

RESULTS

Energy source for motility

Comparison of Motility of Mutants Defective in Oxidative Phosphorylation and of Their Parents. Can ATP serve as the energy source for motility directly, or must it be first converted into the intermediate of oxidative phosphorylation? To answer this we used conditions where the intermediate of oxidative phosphorylation presumably cannot form: formation by means of electron transport to oxygen was blocked by using anaerobic conditions, and formation from ATP was blocked by using mutants unable to carry out that step. ATP synthesis, however, was allowed to occur by means of glycolysis.

Such mutants defective in oxidative phosphorylation are the *E. coli unc*A and ATPase minus (RV5) strains and the *S. typhimurium etc* strain described in *Materials and Methods*. They have recently been extensively used to determine the source of energy for transport of various amino acids (8, 9) and sugars (10). *These mutants, unlike their parents, quickly became nonmotile under anaerobic conditions (to prevent electron transport) even when given substrates that can generate ATP anaerobically*, such as D-glucose or D-galactose (Table 1). Fig. 1 documents this difference between mutant and parent with photomicrographs (5 second exposures). Swimming cells

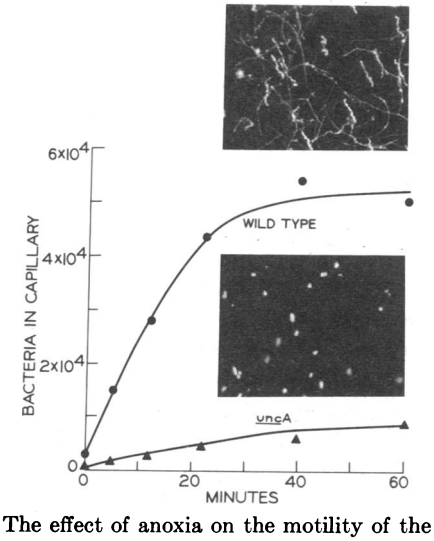

FIG. 1. The effect of anoxia on the motility of the *unc*A mutant (AN120) and its parent (AN180) in 10 mM D-glucose as measured by their ability to swim into a capillary tube. See anaerobic motility assay in *Materials and Methods*. Bacteria were grown on tryptone broth. At the same cell concentration (10^9 cells per ml) and conditions, a paralyzed strain, M526 (34), gave 9200 cells accumulating per capillary in 1 hr. Thus the accumulation of *unc*A can be attributed to some phenomenon other than motility. In an aerobic motility assay time course (with quadruplicate determinations), there was no significant difference between the *unc*A mutant and its parent. *Inserts* are dark field photomicrographs showing the effect of anoxia on the motility of the parental strain (AN180), *top*, and the *unc*A mutant (AN120), *bottom*, under the same conditions. The exposure time was 5 sec (magnification ×74).

appear as tracks while nonmotile cells appear as spots. Fig. 1 also compares the motility of an anaerobic mutant and its parent as measured by their ability to swim into a capillary tube lacking attractant, i.e., containing the same medium as in the bacterial suspension.

The failure of the mutants to swim anaerobically when provided with sugars cannot be attributed to a failure to extensively metabolize the sugars anaerobically or a failure to produce ATP anaerobically. For the *unc*A mutant and its parent, the rate of D-glucose disappearance (μmol/g of cells, dry weight per min) was, respectively, 0.18 and 0.15, aerobically, and 0.15 and 0.22, anaerobically, and the ATP concentration (μmole/g of cells, dry weight) at 40 min was 7.5, 6.0, 7.3, and 5.9 in that same order. For both mutant and parent, CO_2 production aerobically was about 75% of the D-glucose metabolized, and anaerobically, 30%. Less than 3% of the D-glucose metabolized was found to be phosphorylated sugars.

Energy sources that cannot be metabolized anaerobically, such as D-lactate or succinate, of course, do not allow motility anaerobically by either mutants or parents (Table 1), since such energy sources do not provide energy anaerobically.

Thus the energy for motility is presumably coupled directly to some intermediate of oxidative phosphorylation and ATP can serve as an energy source for motility only by driving the Mg^{++},Ca^{++}-ATPase of oxidative phosphorylation in the reverse direction to form this intermediate.

Effect of CCCP on Motility. CCCP has been shown to uncouple oxidative phosphorylation in intact bacteria (25). If the intermediate of oxidative phosphorylation is required for motility, CCCP should paralyze the bacteria, even in the presence of an energy source such as D-glucose that can generate ATP by substrate-level phosphorylation.

This was found to be the case. As observed in the microscope in the presence of 1 μM CCCP, motility with or without 10 mM D-glucose or D-lactate was completely and immediately (less than 10 sec) inhibited, and CCCP had a pronounced inhibitory effect as low as 0.01 μM. This effect of CCCP is documented in Fig. 2 where the ability to swim into a capillary tube is measured (motility assay). Yet the level of ATP in the presence of D-glucose (10 mM) and CCCP (10 μM) was 70% of that without CCCP at 5 min. At 40 min the ATP levels had decreased only to 30%.

We have also tried various other inhibitors but will not report on them here because much higher concentrations were required to inhibit oxidative phosphorylation and (or) electron transport, and their effects in intact bacteria are not as well known. In general the results were, nevertheless, in good agreement with that reported above for CCCP. Reports of such work are, or will be, available [Master's Degree Thesis, J. J. Gargus, Case Western Reserve University, 1973; Ph.D. Thesis, S. H. Larsen, University of Wisconsin, in preparation; see also the work of Faust and Doetsch (16)].

Thus the integrity of the intermediate of oxidative phosphorylation is required for motility. ATP alone is not sufficient to power motility.

Effect of Arsenate on Motility. Arsenate is known to reduce ATP levels in bacteria (7). Is motility still possible in the presence of arsenate, if substrates that support electron transport are present?

Bacteria washed and incubated at 25° in arsenate medium gradually became entirely nonmotile by about 2 hr, but the addition of an electron donor caused an immediate, full restoration of motility as observed in the microscope. D-Lactate or succinate (10 mM) or phenazine metosulfate (1 μM) with ascorbate (10 mM) all served as such electron donors. This was found true for all three mutants as well as wild-type strains. This is documented with a motility assay for a wild-type strain, 20SOK$^-$, grown in glycerol minimal medium: the accumulation in capillaries was 2700 and 470 at 1 hr with and without D-lactate, respectively, in the presence of arsenate. Control cells without arsenate gave accumulations of 2700 and 1900 with and without D-lactate. (See legend to Fig. 1 for explanation of accumulation of nonmotile cells in arsenate without lactate.) Addition of 10 mM D-glucose to the nonmotile cells in arsenate had no effect, presumably because arsenate blocks the metabolism of D-glucose. Bacteria that had never been exposed to arsenate remained motile for over 24 hr with or without (14) an added energy source.

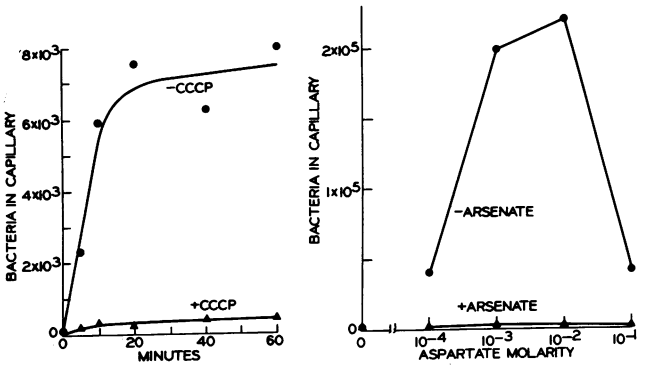

FIG. 2 (*left*). The effect of CCCP on the motility of a wild-type strain (AW574) as measured in the aerobic motility assay described in *Materials and Methods*. Bacteria were grown in tryptone broth. The bacterial suspension contained 2×10^7 cells per ml. The low level of accumulation of cells with CCCP was not due to motility; see legend to Fig. 1. When present, CCCP was 10 μM in both the bacterial suspension and the capillary. The CCCP was dissolved in ethanol; the final concentration of ethanol in the assay, 1%, was found to not inhibit motility. There was no loss in viability.

FIG. 3 (*right*). The effect of arsenate on chemotaxis toward L-aspartate by a wild-type strain (20SOK$^-$). See chemotaxis assay in *Materials and Methods*. Bacteria were grown on glycerol minimal medium. The washed cells were incubated at 25° for 2 hr, DL-lactate was added to 10 mM, and the assay was immediately started. The bacterial suspension contained 2×10^7 cells per ml. Assay time was 1 hr. Arsenate was present in both the capillary and the bacterial suspension and caused no loss in viability.

As a result of arsenate treatment, ATP dropped from 3.80 μmol/g of cells, dry weight, to an undetectable level, <0.01, and remained that low for 2 hr. At 2 hr D-lactate was added to 30 mM and still no ATP was measurable for at least 1 hr.

Thus motility is possible in the virtual absence of ATP so long as the energy of electron transport is available.

Energy source for chemotaxis

Effect of Arsenate on Chemotaxis. Whereas motility was fully restored in cells washed in arsenate by the addition of D-lactate, it was found that chemotaxis was completely missing in these motile bacteria. Fig. 3 shows the typical (19)

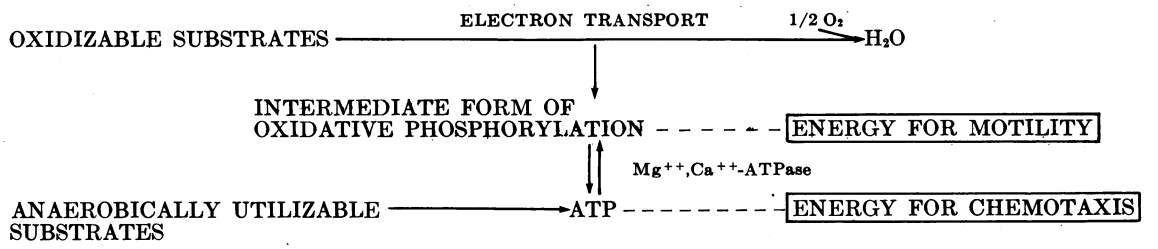

FIG. 4. Energy sources for motility and chemotaxis. (The role of ATP in chemotaxis is indicated by the fact that arsenate eliminates both ATP and chemotaxis, see *Results.*) Since chemotaxis is possible only when there is motility, chemotaxis of course also requires the intermediate of oxidative phosphorylation. See footnote ¶.

chemotactic response to L-asparatate by untreated cells and the failure of arsenate-treated cells to respond. The same lack of chemotactic response was obtained with three wild-type strains of *E. coli* K12: 20SOK$^-$, AW574, and B14. Exactly the same negative response was obtained for all attractants tried, including L-serine, D-glucose, D-galactose, and the non-metabolizable DL-α-methylaspartate, which are all detected by different chemoreceptors (20, 26). When these same cells in arsenate were washed back into medium lacking arsenate (chemotaxis medium), chemotaxis was fully restored, and hence arsenate did not irreversibly harm the chemotactic machinery.

Thus arsenate blocks chemotaxis but allows motility. Some part of the chemotaxis mechanism is inhibited by arsenate, or else ATP or one of its products is required for chemotaxis.

DISCUSSION

Our conclusions are summarized in Fig. 4.

Energy source for motility

The following three lines of evidence have been presented that the energy source for motility in bacteria is the intermediate of oxidative phosphorylation, not ATP. (*i*) Mutants blocked in the "backing up" of ATP into the intermediate of oxidative phosphorylation (see Fig. 4) are paralyzed when they are anaerobic even though they do generate ATP. (*ii*) CCCP, an uncoupler of oxidative phosphorylation, paralyzes bacteria even though ATP remains present. (*iii*) Arsenate brings about the virtual absence of ATP but allows motility if an oxidizable substrate is added.

The present finding presents a striking contrast to the energy requirements found for motility in eukaryotes where ATP is directly utilized to power contraction of muscle (1) and beating of cilia (2) and flagella (3). It will be interesting to see if there is any chemomechanical coupling in eukaryotes that utilizes the intermediate of oxidative phosphorylation rather than ATP directly. While mutants may be useful in some organisms, the use of CCCP and arsenate may be generally applicable for this purpose.

ATPase has never been detected in bacterial flagella (27–30); this absence can now be explained by our finding that ATP is not directly involved in powering bacterial flagella.

The location of the chemomechanical coupling appears to be in the cytoplasmic membrane since that is the site of oxidative phosphorylation and electron transport in bacteria. Also the base of the bacterial flagellum is known to be in intimate contact with the cytoplasmic membrane (30).

A major question that remains is how the intermediate of oxidative phosphorylation is used to drive the flagellum. Paralyzed (*mot*) mutants (29, 31) are presumably defective in this chemomechanical coupling and may be used to provide further insight into the mechanism.

Berg and Anderson (32) have recently reviewed the considerable evidence in favor of the idea that the bacterial flagellum rotates by means of a "biological motor" at the base. They noted that this motor could in principle be driven by crossbridges of the kind found in skeletal muscle; however, since the energy source is different, the mechanism for movement of such a motor might also be different.

Energy source for chemotaxis

The fact that arsenate-treated bacteria swim normally but fail to carry out chemotaxis toward any attractant tried shows that something additional is required for chemotaxis—presumably ATP or one of its many products.

It was previously known that motility is not sufficient for obtaining chemotaxis: mutants (*che*) have been described that are fully motile but fail to carry out any chemotaxis (33), and L-methionine is required for chemotaxis but not for motility (20, 34).

A likely function of ATP in chemotaxis is the activation of L-methionine to *S*-adenosyl-methionine. Some evidence that this reaction is required for chemotaxis has been presented (35). L-Methionine is unable to activate chemotaxis if arsenate is added (data not shown), which suggests that ATP may be required for L-methionine to act. The present results can thus be viewed as supplying further, though indirect, evidence for a function of *S*-adenosyl-methionine in chemotaxis.

Note Added in Proof. Bacterial flagella can rotate both counter-clockwise and clockwise [Silverman, M. & Simon, M. (1974) *Nature*, in press]. Arsenate-treated cells, depleted of ATP, still show both directions of rotation when D-lactate is added; CCCP eliminates both. Hence, the energy source for both is the intermediate of oxidative phosphorylation, not ATP directly.

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