Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10084–10089, September 1996
Biochemistry

Behavioral responses of *Escherichia coli* to changes in redox potential

(redox taxis/electron transport/proton motive force)

VYACHESLAV A. BESPALOV*†, IGOR B. ZHULIN*‡, AND BARRY L. TAYLOR*§

*Department of Microbiology and Molecular Genetics and §Center for Molecular Biology and Gene Therapy, School of Medicine, Loma Linda University, Loma Linda, CA 92350

Communicated by Howard C. Berg, Harvard University, Cambridge, MA, June 25, 1996 (received for review March 18, 1996)

ABSTRACT **_Escherichia coli_ bacteria sensed the redox state in their surroundings and they swam to a niche that had a preferred reduction potential. In a spatial redox gradient of benzoquinone/benzoquinol, _E. coli_ cells migrated to form a sharply defined band. Bacteria swimming out of either face of the band tumbled and returned to the preferred conditions at the site of the band. This behavioral response was named redox taxis. Redox molecules, such as substituted quinones, that elicited redox taxis, interact with the bacterial electron transport system, thereby altering electron transport and the proton motive force. The magnitude of the behavioral response was dependent on the reduction potential of the chemoeffector. The Tsr, Tar, Trg, Tap, and CheR proteins, which have a role in chemotaxis, were not essential for redox taxis. A _cheB_ mutant had inverted responses in redox taxis, as previously demonstrated in aerotaxis. A model is proposed in which a redox effector molecule perturbs the electron transport system, and an unknown sensor in the membrane detects changes in the proton motive force or the redox status of the electron transport system, and transduces this information into a signal that regulates phosphorylation of the CheA protein. A similar mechanism has been proposed for aerotaxis. Redox taxis may play an important role in the distribution of bacterial species in natural environments.**

Bacteria are guided to favorable niches in their surroundings by chemotaxis to diverse stimuli including amino acids and carbohydrates (1), metal ions (2), and oxygen and other electron acceptors (1, 3). They also show a behavioral response to changes in intense light (3, 4), temperature (5), pH (6, 7), and osmotic strength (8). Behavioral responses to these environmental stimuli allow bacteria to find optimal conditions for growth in their surroundings.

In *Escherichia coli*, the majority of chemoeffectors are sensed by transmembrane receptors called methyl-accepting chemotaxis proteins (reviewed in refs. 9 and 10). These chemoreceptors modulate a phosphorylation cascade that involves the CheA, CheW, and CheY proteins (11). There are alternative chemotaxis pathways that are independent of the methyl-accepting chemotaxis proteins. The most extensively studied of the alternative sensory transduction pathways in *E. coli* are the pathways for aerotaxis and sugars of the phosphotransferase system (12–14). Aerotaxis is the behavioral response that guides bacteria to the concentration of oxygen that is optimal for growth of the species. Oxygen is reduced by the electron transport system and the bacteria detect and respond to the changes in the respiratory system. Current evidence suggests that the bacteria sense a change in the proton motive force (15–18), although the tight coupling of H^+ translocation to electron transport leaves open a possibility that the bacteria sense changes in electron transport, such as the redox state of the components of the respiratory system. The proton motive force across the bacterial cytoplasmic membrane is a primary form of energy in the cell (reviewed in ref. 19); sensing of changes in the proton motive force enables bacteria to find optimal conditions for energy generation. Phototaxis (20–23) and taxis toward electron acceptors (3) in various bacterial species are additional examples of behaviors mediated by the proton motive force and a sensor that is not a methyl-accepting chemotaxis protein (3, 24, 25).

In this study we present evidence that, in addition to known behavioral responses, *E. coli* senses the redox state in its surroundings. The term redox taxis has been adopted for this behavior. The mechanism of redox taxis is compared with the mechanism of aerotaxis.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The strains used are derivatives of *E. coli* K-12. Bacteria were grown in Luria-Bertani broth at 35°C to OD_{600} = 0.4–0.6. Cells were washed twice and resuspended in chemotaxis buffer (26) supplemented with 20 mM sodium lactate.

Spatial Assay for Redox Taxis. A redox gradient of reduced/oxidized quinone was formed on a microscope slide as described (27). A plug of agarose, containing 100 μM 1,4-benzoquinone and 2 mM potassium ferricyanide to maintain the quinone in the oxidized form, was inserted into a suspension of *E. coli* MM335 (6×10^8 cells/ml) containing 100 μM 1,4-benzoquinol (reduced form of the quinone). The preparation was formed and maintained under anaerobic conditions. Formation of a tactic band of bacteria around the plug was observed and recorded via a dark-field video microscope. Spatial gradients of ferricyanide and of benzoquinol were used as negative controls.

Temporal Assay for Redox Taxis. A 9 μl drop of diluted (2×10^7 cells/ml) bacterial suspension was placed on a microscope slide and the unstimulated motility pattern of the bacteria was observed using a dark-field video microscope. The compound to be tested (1 μl) was added to the suspension to give a final concentration of 20 μM (for adaptation time measurements) or as shown (for threshold concentration measurements). Changes in motility were recorded. Tumbling frequency was determined by computerized motion analysis using a VP110 video processor (Motion Analysis, Santa Rosa, CA) and a program developed using EXPERTVISION software (28). To measure redox taxis under anaerobic conditions, experiments were performed in a microchamber ventilated with humidified nitrogen as previously described (16).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†Present address: Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov 410015, Russia.
‡To whom reprint requests should be addressed. e-mail: iZhulin@ccmail.llu.edu at internetmail.

Measurement of Membrane Potential. The membrane potential was determined using a permeating lipophilic cation, tetraphenyl phosphonium (18). The tetraphenyl phosphonium concentration in the external medium was measured with a tetraphenyl phosphonium-selective electrode constructed according to Kamo *et al.* (29) using a Kwik-Tip Ag/AgCl half cell electrode (World Precision Instruments, Sarasota, FL). The tetraphenyl phosphonium-selective electrode and a semimicro calomel reference electrode (Orion, Boston) were connected to an ion meter (Corning) and a MacLab MKIII data recording system (Analog Digital Instruments, Milford, MA). All measurements were carried out in a 10 ml closed vessel at 30°C and pH 7.5. Final cell concentration was 8×10^9 cells/ml. The cells were made permeable to tetraphenyl phosphonium by treatment with EDTA as described (30). The efficiency of the permeabilization was determined by assaying the loss of motility after addition of Triton X-100.

Measurement of Oxygen Concentration. Respiration rates of bacterial suspensions were measured using a Clark-type electrode and an oxygen monitor (Yellow Springs Instruments) that was connected to the data recording system. The oxygen concentration in the bacterial suspension during the measurements of the membrane potential was monitored using a needle oxygen electrode and a chemical microsensor (Diamond General, Ann Arbor, MI).

RESULTS

Behavioral Response in a Redox Gradient. *E. coli* cells placed in a spatial oxygen gradient migrate to a sharply defined band (Fig. 1*a*) because the bacteria are attracted to a preferred concentration of oxygen and repelled by both higher concentrations of oxygen and anoxia (31). A similar band was formed in a redox gradient under anaerobic conditions (Fig. 1*b*). The redox gradient was formed by inserting a plug of agarose containing 1,4-benzoquinone (oxidized form) into a bacterial suspension containing an equamolar concentration of 1,4-benzoquinol (reduced form). This created a gradient of oxidized quinone in the bacterial suspension. The plug also contained potassium ferricyanide to maintain benzoquinone in the oxidized state. The total quinone concentration (reduced plus oxidized) was constant throughout the gradient. The observation that *E. coli* cells migrated to a sharply defined band (Fig. 1*b*) suggested that the bacteria were attracted to a preferred reduction potential of the quinone in the medium. Microscopic observations using dark-field illumination revealed that the cells in the redox tactic band were highly motile even though no terminal electron acceptor was present in the medium. Cells swimming out of the band in either direction tumbled immediately and returned to the band. This is similar to previous observations in aerotactic bands (31). In a spatial gradient of potassium ferricyanide alone, bacteria did not form a band, even though there was a gradient of reduction potential in the medium.

Redox Taxis Is Mediated by the Molecules Interacting with the Electron Transport System. A temporal assay was used to investigate the redox tactic response and to screen redox molecules, such as substituted quinones, for the ability to elicit an attractant (smooth swimming) or repellent (constantly tumbling) response (Table 1). Under aerobic conditions, the oxidized quinones tested were repellents. The reduced forms of the quinones did not elicit a tactic response at concentrations up to 2 orders of magnitude higher than the concentration of the oxidized form that elicited a response. The reductants, 2-mercaptoethanol, dithiothreitol, and sodium borohydride elicited a brief smooth swimming response (Table 1).

Not all redox molecules were effectors for redox taxis (Table 1) and this facilitated investigation of the initial transduction event in redox taxis. In a spatial quinone/quinol gradient (Fig. 1*b*), the band of bacteria moved closer to the plug over time.

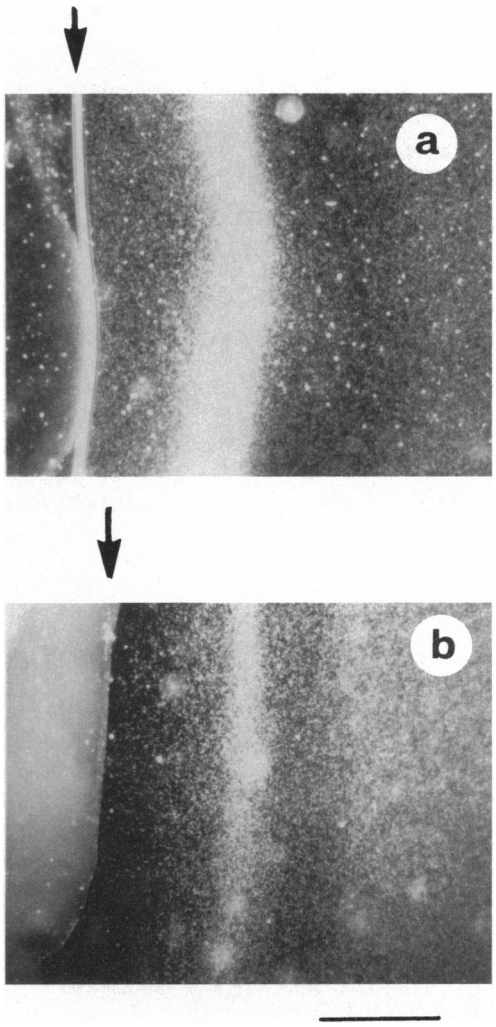

FIG. 1. Comparison of *E. coli* migration in oxygen and redox gradients. (*a*) An oxygen gradient was formed in a suspension of *E. coli* MM335 (wild type for chemotaxis) on a microscope slide covered with a coverslip, by oxygen diffusion through the air/suspension interface (arrow), and by oxygen consumption by the respiring cells (31). The preparation was photographed after 10 min. (*b*) A redox gradient of benzoquinone/benzoquinol was formed on a microscope slide (see *Materials and Methods*). The preparation was maintained under anaerobic conditions and photographed after 10 min. An arrow marks the interface between the plug and the cell suspension. (Bar = 0.5 mm.)

Concurrently, the quinone solution in the vicinity of the band changed from yellow (oxidized form) to colorless (reduced form). This suggested that 1,4-benzoquinone was reduced by the bacteria. To confirm this, reduction of both potassium ferricyanide and 1,4-benzoquinone by the bacterial cells was measured by monitoring changes in absorbance at 422 nm (maximum for ferricyanide) and 430 nm (maximum for benzoquinone). Benzoquinone was readily reduced by the cells (data not shown).

In the electron transport system of *E. coli* (33), ubiquinone (a substituted 1,4-benzoquinone) and menaquinone (a substituted 1,4-naphthoquinone) link the various dehydrogenase complexes to the terminal oxidases (under aerobic growth) or terminal reductases (under anaerobic growth). Exogenous benzoquinones and naphthoquinones can divert electrons from the electron transport system by interacting directly with the respiratory dehydrogenases (34) or terminal oxidases (35). In redox taxis, benzoquinone diverted electrons from the electron transport system under aerobic conditions. Oxygen

Table 1. Behavioral responses of *E. coli* to redox compounds

Compound	Reduction potential† mV	Behavioral response* Threshold, μM	Behavioral response* Adaptation time, s
1,4-Benzoquinone (BQ)			
2,6-Dichloro-BQ	ND	1	>250
BQ	+99	2	>250
2-Methyl-BQ	+23	2	150
2,6-Dimethyl-BQ	−80	4	60
2,3-Dimethoxy-5-methyl-BQ	ND	6	50
Tetramethyl-BQ	−240	40	NR
1,4-Naphthoquinone (NQ)			
5-Hydroxy-NQ	−93	0.2	150
NQ	−140	1	110
5-Hydroxy-2-methyl-NQ	−156	2	70
2-Methyl-NQ	−203	5	60
2-Hydroxy-NQ	−415	400	NR
Reductant			
2-Mercaptoethanol	ND	1	15
Dithiothreitol	ND	1	15
Sodium borohydride	ND	0.1	15
Other redox compounds:			
Methyl viologen	−446	NR	NR
Methylene blue	+11	NR	NR
Potassium ferricyanide	+420	NR	NR

ND, not determined; NR, no response.

*The behavioral responses of *E. coli* MM335 were measured in a temporal gradient assay. The oxidized quinones tested were repellents and the reductants were attractants. The time interval was determined for 50% of the cell population to return to prestimulus behavior after the addition of the compound to be tested (final concentration, 20 μM). This is defined as the adaptation time. Quinones were dissolved in dimethyl sulfoxide or in ethanol. The final concentration of dimethyl sulfoxide and ethanol in water solutions in the assay was <0.01% and did not cause a behavioral response in *E. coli*.

†Data from Wardman (32).

consumption by the bacteria was decreased, and ceased at high concentrations of benzoquinone (Fig. 2*d*). Other redox compounds that elicited a repellent response (Table 1) were also reduced by *E. coli* cells, and they decreased oxygen consumption by the bacteria. In contrast, ferricyanide, which does not elicit redox taxis, was not reduced by *E. coli* and did not affect oxygen uptake by the bacteria (data not shown). This probably reflects the impermeability of the *E. coli* membrane to ferricyanide. Methylene blue and methyl viologen did not elicit taxis (Table 1) and they were not reduced and oxidized, respectively, by the cells.

Magnitude of Redox Taxis Is Dependent on Reduction Potential of the Signal Molecule. In Table 1 there was a direct correlation between the magnitude of the behavioral response and the reduction potential (electron affinity) of the quinone that elicited the response. We conclude that the stronger the ability of a molecule to divert electrons from the electron transport system, the more potent it is as an effector for a behavioral response.

Reduction/Oxidation Is a Signal for the Behavioral Response. *E. coli* responded to the oxidation or reduction of a quinone *in situ*, where the total quinone/quinol concentration remained constant. Addition of reduced 1,4-benzoquinol to the cells under aerobic conditions had no effect on bacterial behavior (Fig. 2*a*), electron transport (Fig. 2*c*), or the proton motive force (Fig. 2*e*). However, when potassium ferricyanide was added to the suspension to oxidize the quinol *in situ*, the diversion of electrons to oxidized benzoquinone inhibited respiration (Fig. 2*c*) and the proton motive force (Fig. 2*e*), and concurrently elicited a constantly tumbling response (Fig. 2*a*). In the absence of the quinol, ferricyanide did not elicit a response.

Reduction of a quinone *in situ* had the opposite effect. The diversion of electrons (inhibition of respiration) ceased when benzoquinone was reduced by mercaptoethanol (Fig. 2*d*); the proton motive force was restored (Fig. 2*f*) and the cells ceased their constant tumbling (Fig. 2*b*) and swam smoothly for ≈2 min. When added alone, mercaptoethanol caused a 15-sec smooth swimming response.

Correlation of Proton Motive Force with Redox Taxis. In aerobic *E. coli*, oxidized quinones were repellents (Table 1). Cells grown anaerobically with nitrate responded to the quinone similarly to the aerobically grown cells—i.e., the oxidized quinones caused a tumbling response. However, if the aerobically grown bacteria were first exposed to anoxia, oxidized quinones were attractants. In a temporal behavioral assay under anaerobic conditions, the cells showed a 25-sec smooth swimming response and a transient increase in cell speed upon addition of 5 μM 1,4-benzoquinone.

The evidence presented above indicates that under aerobic conditions or during anaerobic respiration a redox tactic response is initiated when electrons are diverted from the electron transport system to reduce the signal molecule. This decreases both electron transport and the proton motive force (Fig. 2). It is likely that bacteria sense and respond to changes in proton motive force or in the redox status of the components of the electron transport system. The results obtained in anoxic *E. coli* were consistent with this hypothesis. When aerobically grown *E. coli* cells were exposed to anoxia, motility dramatically decreased due to the absence of electron transport and a consequent decrease in the proton motive force which supplies the energy for the *E. coli* flagellar motors (37). It was assumed that under anoxic conditions (where there is no respiration), addition of oxidized (but not reduced) quinone would permit limited electron transport, and an increase in the proton motive force by acting as an electron acceptor for the respiratory dehydrogenase complexes. Fig. 3 confirmed that addition of the oxidized quinone to bacterial cells under anaerobic conditions resulted in a transient increase in the proton motive force. Addition of reduced benzoquinol or an impermeable redox compound, potassium ferricyanide, did not result in changes in the proton motive force or bacterial behavior (data not shown). Thus, in both aerobic (Fig. 2) and anaerobic (Fig. 3), conditions negative redox taxis coincides with a decrease in the proton motive force and positive redox taxis coincides with an increase in the proton motive force.

Redox Taxis Is Independent of Methylated Chemotaxis Proteins. In most chemotactic responses, adaptation of *E .coli* is dependent on the methylation of specific chemoreceptors by the CheR methyl transferase (reviewed in refs. 9 and 10). In contrast, adaptation during aerotaxis is methylation independent (12). Our results demonstrate that redox taxis does not require methylation (CheR) or methyl-accepting chemotaxis proteins (Tsr, Tar, Trg, and Tap) (Table 2). The inverted response of the *cheB* mutant to both an oxidant and a reductant (Table 2) is another similarity to aerotaxis (39).

DISCUSSION

A Model for Signaling in Redox Taxis. This investigation documents a novel bacterial behavior in which cells monitor their redox environment by responding to the reduction or oxidation of a chemical by the bacterial electron transport system. Several types of redox chemicals were shown to cause a behavioral response in *E. coli* (Table 1).

Reductants, such as mercaptoethanol and sodium borohydride, caused a brief smooth swimming response under aerobic conditions and no response under anaerobic conditions. Since the reductants readily reduce quinones in solution (Fig. 2), it is possible that they may have a similar effect on the naturally

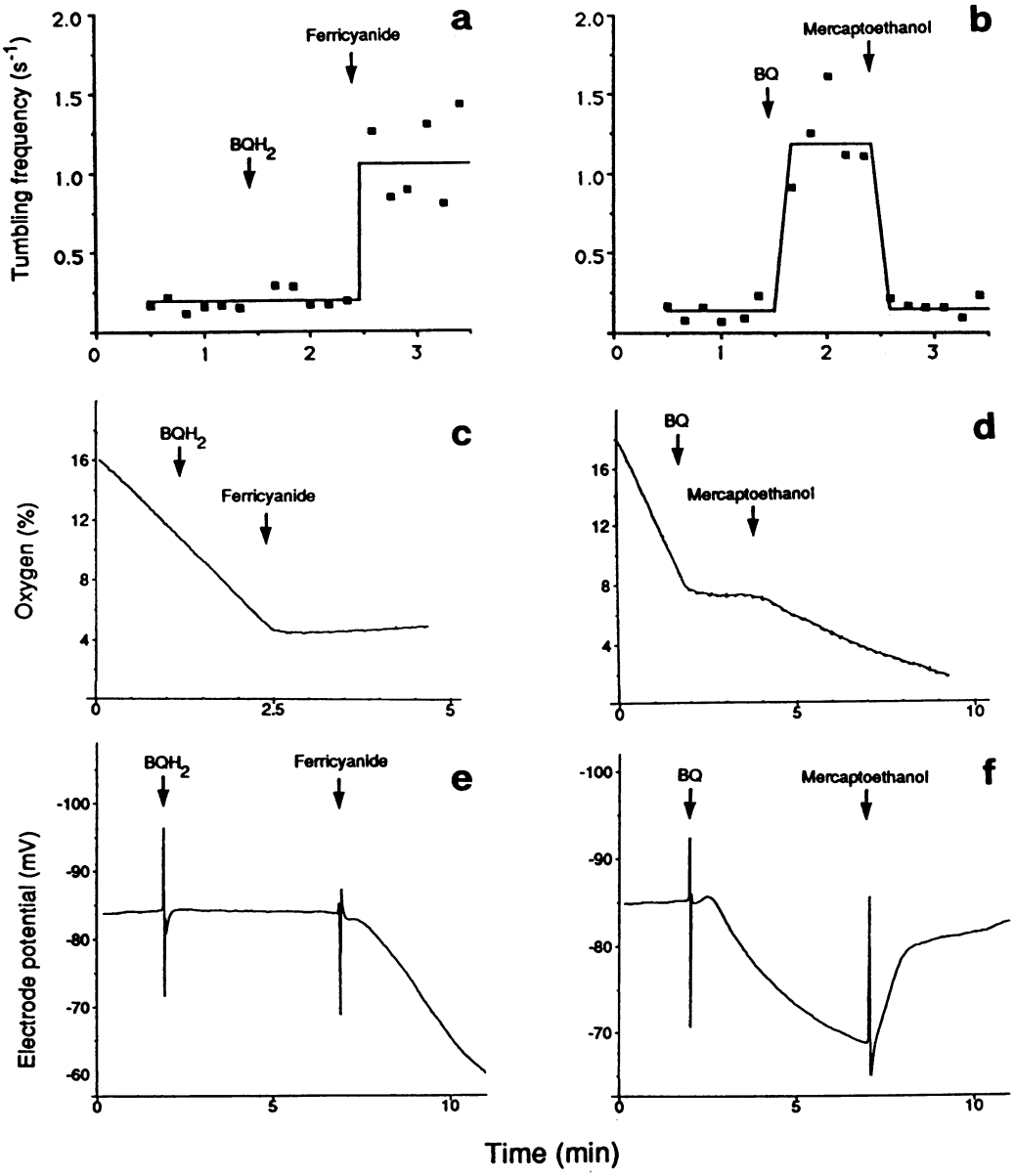

FIG. 2. Responses of *E. coli* MM335 to the reduction and oxidation of an exogenous quinone under aerobic conditions. (*a*, *c*, and *e*) The addition of 1,4-benzoquinol (BQH_2) was followed by potassium ferricyanide to oxidize the quinol. (*b*, *d*, and *f*) The addition of 1,4-benzoquinone (BQ) was followed by mercaptoethanol to reduce the quinone. (*a* and *b*) Behavioral response to oxidation (*a*) or reduction (*b*) of the quinone. Tumbling frequency was determined by computerized motion analysis. Cell concentration: 1×10^8 cells/ml. Final concentrations: BQ and BQH_2, 10 μM; potassium ferricyanide and mercaptoethanol, 200 μM. (*c* and *d*) Changes in oxygen uptake. Oxygen concentration was measured with a Clark-type oxygen electrode. Cell concentration: 4×10^8 cells/ml. Final concentrations: BQ and BQH_2, 100 μM; potassium ferricyanide and mercaptoethanol, 200 μM. (*e* and *f*) The proton motive force was monitored as membrane potential at pH 7.5, where the chemical proton gradient is zero (36), using a tetraphenyl phosphonium-selective electrode. The electrode traces commenced after equilibration of tetraphenyl phosphonium between the extra- and intracellular spaces. Cell concentration: 8×10^9 cells/ml. Because the higher cell concentration rapidly reduced BQ to BQH_2, concentrations were increased to 1 mM for BQ and BQH_2 and 2 mM for potassium ferricyanide and mercaptoethanol.

occurring quinones in the bacterial electron transport system. Under aerobic conditions, ubiquinone is predominantly in the oxidized form, and thus addition of a reductant may enhance electron transport between dehydrogenases and terminal oxidase complexes.

The strongest redox tactic responses were observed for quinones that are analogues of ubiquinone and menaquinone. Under anoxic conditions, the exogenous quinones permit limited electron transport and generation of the proton motive force (Fig. 3), presumably via dehydrogenase complexes. Under aerobic conditions and during anaerobic respiration, exogenous quinones interrupt electron transport between dehydrogenases and terminal oxidases or reductases, competing for electrons with naturally occurring quinones, and thus inhibit respiration and decrease the proton motive force (Fig. 2).

Our results are consistent with a model in which a change in the proton motive force is the signal transduction event that triggers the behavioral response. But since the proton motive force is tightly coupled to electron transport, our results are also consistent with a model in which the critical sensory transduction event is a change in the redox state of a component of the electron transport system. Redox sensing has previously been implicated in a photophobic response in cyanobacteria (40) and in controlling the activity of the light-harvesting chlorophyll protein kinase in a unicellular green alga (41). Further investigations are necessary to dis-

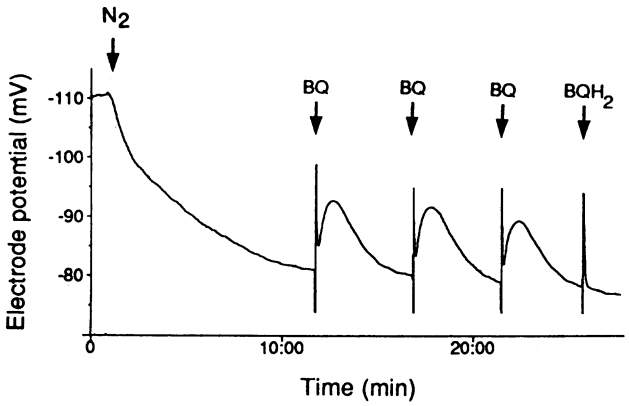

FIG. 3. Changes in the proton motive force upon stimulation of anoxic *E. coli* MM335 with 1,4-benzoquinone. Conditions for the proton motive force measurement are described in the legend to Fig. 2. The cell suspension was sparged with 21% oxygen to maintain aerobic conditions and then with 100% nitrogen (N_2) (arrow) to achieve anoxic conditions. Benzoquinone (BQ) and benzoquinol (BQH_2) were added at final concentrations 200 μM (arrows).

tinguish between changes in the proton motive force and in the redox state as the essential signal transduction event.

We propose a model for redox taxis in which a sensor in the membrane detects the change in redox state or the proton motive force and generates a signal that modulates phosphorylation of the CheA protein, thereby regulating the flagellar motors that control the bacterial behavior. This model is similar to a proposed mechanism for bacterial aerotaxis (13, 18, 25). As in aerotaxis, a signal molecule in redox taxis must be reduced (oxidized) in order to cause a behavioral response. Like aerotaxis, redox taxis does not require the presence of a known chemotaxis receptor, and adaptation is methylation-independent (Table 2). Both aerotaxis and redox taxis are inverted in a *cheB* mutant. Previous findings in this laboratory demonstrated that the central chemotaxis regulator, CheA, interacts with the aerotaxis sensor (42). The similarities between redox taxis and aerotaxis suggest that a common sensor may function for redox taxis and aerotaxis.

Changes in the redox potential of the environment have a great impact on a living cell. Adaptation of bacteria to such changes at the level of gene expression is well documented for ArcA/ArcB, FixL/FixJ, FNR, NifL/NifA, and similar systems (reviewed in refs. 43 and 44). However, the exact mechanism of redox sensing remains unknown. Most of the sensor proteins of the two- or one-component regulatory systems that control expression of aerobic or anaerobic metabolism contain heme or an iron sulfur center. These cofactors may sense oxygen *per se* (45) or a reduction potential (46). Redox taxis should be a productive system for further investigating the basic principles of redox sensing in microorganisms.

Possible Ecological Role of Redox Taxis. Exponentially growing aerobic bacteria consume oxygen and reduce their surroundings. As the bacterial density increases, continued growth is endangered by this conditioning of the surroundings. Redox taxis and aerotaxis can disperse crowded bacteria before they become trapped in a hypoxic, reduced environment. A response to redox potential also may be important to bacteria inhabiting the oxic-anoxic transition zones found in soil, in the rhizosphere, in sediment-water interfaces, and in some water columns (47, 48). We have previously demonstrated sensing by a nitrogen-fixing bacterium, *Azospirillum brasilense*, of a redox gradient of an artificial electron acceptor (27). Redox taxis could guide individual bacterial species in anoxic zones to the optimal redox potential for hydrogen utilization, nitrogen fixation, or for biogeochemical cycling of sulfur, iron and carbon. If so, it is likely that redox taxis is widespread in bacteria, and that there are many additional effectors for redox taxis.

Finally, the repellent responses observed in *E. coli* correlated not with the ability of quinones to produce reactive oxygen derivatives (data not shown) but with their capacity to divert electrons from the electron transport system (Table 1). Cytotoxicity of quinones as anticancer and antibacterial drugs is widely believed to be due to production of toxic oxygen derivatives (49). In light of our findings, it would be interesting to investigate whether the cytotoxic action of quinones is due to their ability to act as inhibitors of the electron transport system (34).

Table 2. Response to redox compounds by *E. coli* chemotaxis mutants

Strain (relevant phenotype)	Prestimulus behavior	Response to 1,4-BQ	Response to $NaHB_4$	Source (ref.)
MM335 (wild type)	Random	Tumbling	Smooth	M. Manson*
AW655 (Tsr)	Random	Tumbling	Smooth	J. Adler (38)
AW656 (Tar)	Random	Tumbling	Smooth	J. Adler (38)
AW701 (Trg)	Random	Tumbling	Smooth	J. Adler (38)
RP1061 (Tap, CheR)	Smooth	Tumbling	Smooth†	J. Parkinson‡
RP4971 (CheB)	Tumbling	Smooth	Tumbling§	J. Parkinson

In a temporal assay, 1,4-benzoquinone (1,4-BQ; 10 μM) or sodium borohydride ($NaHB_4$; 10 μM) was added to a suspension of the designated *E. coli* strains and the response was observed.

*Texas A&M University.

†To analyze a response to sodium borohydride in the smooth swimming *cheR* mutant, the cells were preincubated with the repellent leucine (100 μM) to induce tumbling, followed by addition of sodium borohydride (200 μM).

‡University of Utah.

§To analyze an inverse response to borohydride in the constantly tumbling *cheB* mutant, the cells were preincubated with the attractant serine (10 μM) to induce smooth swimming, followed by addition of borohydride (200 μM).

We thank J. Adler, J. S. Parkinson, and M. D. Manson for providing bacterial strains and M. S. Johnson, R. M. Macnab, and E. A. Magnusson for helpful discussions. This work was supported by Grant GM29481 from the National Institutes of Health (to B.L.T.).

1. Adler, J. (1966) *Science* **153,** 708–716.
2. Zukin, R. S. & Koshland, D. E., Jr. (1976) *Science* **193,** 405–408.
3. Taylor, B. L., Miller, J. B., Warrick, H. M. & Koshland, D. E., Jr. (1979) *J. Bacteriol.* **140,** 567–573.
4. Taylor, B. L. & Koshland, D. E., Jr. (1975) *J. Bacteriol.* **123,** 557–569.
5. Maeda, K. & Imae, Y. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 91–95.
6. Repaske, D. R. & Adler, J. (1981) *J. Bacteriol.* **145,** 1196–1208.
7. Kihara, M. & Macnab, R. M. (1981) *J. Bacteriol.* **145,** 1209–1221.
8. Li, C., Boileau, A. J., Kung, C. & Adler, J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9451–9455.
9. Hazelbauer, G. L. (1992) *Curr. Opin. Struct. Biol.* **2,** 505–510.
10. Parkinson, J. S. (1993) *Cell* **73,** 857–871.
11. Bourret, R. B., Borkovich, K. A. & Simon, M. (1991) *Annu. Rev. Biochem.* **60,** 401–441.
12. Niwano, M. & Taylor, B. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 11–15.
13. Taylor, B. L. (1983) *Trends Biochem. Sci.* **8,** 438–441.
14. Lux, R. Jahres, K., Bettenbrock, K., Parkinson, J. S. & Lengeler, J. W. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11583–11587.
15. Miller, J. B. & Koshland, D. E., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4752- 4756.
16. Laszlo, D. J. & Taylor, B. L. (1981) *J. Bacteriol.* **145,** 990-1001.
17. Shioi, J. & Taylor, B. L. (1984) *J. Biol. Chem.* **259,** 10983–10988.
18. Shioi, J., Tribhuwan, R. C., Berg, S. T. & Taylor, B. L. (1988) *J. Bacteriol.* **170,** 5507–5511.
19. Boyer, P. D., Chance, B., Ernster, L., Mitchell, P., Racker, E. & Slater, E. C. (1977) *Annu. Rev. Biochem.* **46,** 955-1026.
20. Harayama, S. & Iino, T. (1977) *J. Bacteriol.* **131,** 34–41.

21. Armitage, J. P., Ingham, C. & Evans, M. C. W. (1985) *J. Bacteriol.* **161,** 967–972.
22. Baryshev, V. A., Glagolev, A. N. & Skulachev, V. P. (1981) *Nature (London)* **292,** 338–340.
23. Bibikov, S. I., Grishanin, R. N., Kaulen, A. D., Marwan, W., Oesterhelt, D. & Skulachev, V. P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9445–9450.
24. Glagolev, A. N. (1980) *J. Theor. Biol.* **82,** 171–185.
25. Taylor, B. L. (1983) *Annu. Rev. Microbiol.* **37,** 551–573.
26. Adler, J. (1973) *J. Gen. Microbiol.* **74,** 77–91.
27. Grishanin, R. N., Chalmina, I. I. & Zhulin, I. B. (1991) *J. Gen. Microbiol.* **137,** 2781- 2785.
28. Lindbeck, J. C., Goulbourne, E. A., Johnson, M. S. & Taylor, B. L. (1995) *Microbiology* **141,** 2945–2953.
29. Kamo, N., Muratsugu, M., Hongoh, R. & Kobatake, Y. (1979) *J. Membr. Biol.* **49,** 105–121.
30. Szmelcman, S. & Adler J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4387–4391.
31. Shioi, J., Dang, C. V. & Taylor, B. L. (1987) *J. Bacteriol.* **169,** 3118–3123.
32. Wardman, P. (1989) *J. Phys. Chem. Ref. Data* **18,** 1645–1719.
33. Anraku, Y. (1988) *Annu. Rev. Biochem.* **57,** 101–132.
34. Imlay, J. & Fridovich, I. (1992) *Arch. Biochim. Biophys.* **296,** 337–346.
35. Sato-Watanabe, M, Mogi, T., Miyoshi, H., Iwamura, H., Matsushita, K., Adachi, O. & Anraku, Y. (1994) *J. Biol. Chem.* **269,** 28899–28907.
36. Slonczewski, J. L., Rosen, B. P., Alger, J. R. & Macnab, R. M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6271–6275.
37. Larsen, S. H., Adler, J., Gargus, J. J. & Hogg, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1239–1243.
38. Kondoh, H., Ball, C. B. & Adler, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 260- 264.
39. Dang, C. V., Niwano, M., Ryu, J.-I. & Taylor, B. L. (1986) *J. Bacteriol.* **166,** 275–280.
40. Gabai, V. L. & Glagolev, A. N. (1985) *FEMS Microbiol. Lett.* **27,** 351–356.
41. Frid, D., Gal, A., Oettmeier, W., Hauska, G., Berger, S. & Ohad, I. (1992) *J. Biol. Chem.* **267,** 25908–25915.
42. Rowsell, E. H., Smith, J. M., Wolfe, A. & Taylor, B. L. (1995) *J. Bacteriol.* **177,** 6011–6014.
43. Gunsalus, R. P. (1992) *J. Bacteriol.* **174,** 7069–7074.
44. Iuchi, S. & Lin, E. C. C. (1993) *Mol. Microbiol.* **9,** 9–15.
45. Gilles-Gonzalez, M. A. & Gonzalez, G. (1993) *J. Biol. Chem.* **268,** 16293–16297.
46. Unden, G., Trageser, M. & Duchene, A. (1990) *Mol. Microbiol.* **4,** 315–319.
47. Canfield, D. E. & Des Marais, D. J. (1991) *Science* **251,** 1471–1473.
48. Risatti, J. B., Capman, W. C & Stahl, D. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10173–10177.
49. O'Brien, P. J. (1991) *Chem.-Biol. Interact.* **80,** 1–41.