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Effect of Arsenate on Chemotactic Behavior of *Escherichia coli*

TAKAO ARAI†

Departments of Biochemistry and Genetics, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

Escherichia coli cells treated with arsenate cannot tumble. The relationship between cellular adenosine 5'-triphosphate (ATP) level and the ability to tumble has been studied. (i) Cells incubated with arsenate completely lost their tumbling ability, and the cellular ATP level was decreased to less than 0.3 nmol/mg of protein. (ii) Incubation with 10 mM arsenate–1 mM phosphate reduced the cellular ATP level to less than 0.25 nmol/mg of protein. However, the cells were still able to tumble. (iii) Tumbling of the arsenate-treated cells was completely recovered after addition of a slight amount of phosphate, although the ATP level was still as low as 0.2 nmol/mg of protein. (iv) The cellular ATP level of an arsenate-treated *uncA* mutant (Ca^{2+},Mg^{2+}-adenosine triphosphatase defective) was lower than 0.1 nmol/mg of protein even after the addition of 5 mM phosphate. However, tumbling ability was almost completely restored upon addition of the phosphate.

Movement of motile bacteria is affected by environmental conditions such as chemical composition, light intensity, and temperature. Bacteria such as *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* are attracted by certain chemicals and repelled by others. This behavioral response has been called chemotaxis.

Under unstimulated conditions (in the absence of any chemical gradient), cells swim in a straight line for a while and then abruptly change their direction of swimming by tumbling. Bacteria swim by rotating their flagella. Smooth swimming results from counterclockwise rotation of flagella, whereas tumbling is due to clockwise rotation. Tumbling occurs with a constant probability but at random intervals. This probability is modulated by spatial or temporal gradients of chemicals. When cells detect an increasing concentration of attractant (or a decreasing concentration of repellent), the probability of tumbling is markedly decreased. On the other hand, when cells swim toward a higher concentration of repellent (or a lower concentration of attractant) tumbling occurs more frequently. The change in concentration of a chemical stimulus is detected by chemoreceptors, signalled to the flagellum motor apparatus by an unknown mechanism to affect the direction of rotation, counterclockwise or clockwise. The phenomenon of transmitting information of flagella from chemoreceptors has been called sensory transduction. The response to changes in chemical concentration is transient, and the tumbling frequency eventually returns to the unstimulated level. The change in tumbling frequency and the decline in response are called sensory excitation and adaptation, respectively (see 1, 3, 7, and 14 for review).

Adler and Dahl (2) observed that methionine auxotrophs of *E. coli* required L-methionine for both chemotaxis and spontaneous tumbling. It was shown that the methionine functioned via *S*-adenosylmethionine (4–6) and that the methyl group of *S*-adenosylmethionine was transferred to a set of membrane proteins, methyl-accepting chemotaxis proteins (13). Larsen et al. (15) found that chemotaxis was completely inhibited by arsenate treatment, whereas motility was not affected. They suggested that this effect was due to a decrease in the cellular ATP level and that ATP was used for activation of L-methionine to *S*-adenosylmethionine. However, recent studies show that some tumbling mutants still tumble after methionine starvation, whereas arsenate treatment makes all tumbling mutants unable to tumble. From these results, it is thought that ATP itself is required for tumbling, independent of its role in activation of methionine (6, 12, 21).

Little is known about the molecular mechanism of sensory excitation, although it is well known that methylation of methyl-accepting chemotaxis proteins plays an essential role in sensory adaptation. The finding of a methionine requirement for chemotaxis led to the discovery

† Present address: Institute of Basic Medical Sciences, The University of Tsukuba, Niihari-gun, Ibaraki 305, Japan.

of the methylation reaction. Therefore, it is hoped that clarification of the involvement of ATP in the regulation of tumbling may provide important clues for understanding the mechanism of sensory excitation. Using a *cheX* mutant (methyl-accepting chemotaxis protein methyltransferase defective) and an *uncA* mutant (Ca^{2+},Mg^{2+}-ATPase defective), the relationship between the cellular ATP level and the ability to tumble has been investigated. The *cheX* mutant was used for this experiment instead of a wild-type strain, because the mutant shows prolonged and continuous tumbling in the presence of repellent whereas repellent-induced tumbling in the wild type is brief and transient (10). Therefore, the behavior of the mutant is much easier to follow under microscopy than that of the wild-type strain. This study shows that when phosphate was absent in the medium, arsenate-treated cells became unable to tumble while retaining a relatively high ATP level. However, these cells were still able to tumble even at very low ATP levels if a small amount of phosphate was present in the medium.

MATERIALS AND METHODS

Bacterial strains and growth. RP487 (13) is wild type for chemotaxis. RP4080 is a *cheX* mutant isolated by Parkinson (18, 19) from the chemotactically wild-type strain RP470 (closely related to RP487), and it carries the most defective *cheX217* allele (10). AN180 is the parent strain of *uncA* mutant AN120 (8).

Cells were grown in Vogel-Bonner minimal medium (23) with required amino acids and were harvested by centrifugation at an optical density at 590 nm of 0.6 to 0.8.

Observation of tumbling ability. The cells were washed three times with 10 mM PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)] buffer, pH 6.6, and suspended in 5 ml of the same buffer at a final concentration of 1.5 to 2.0 optical density units at 590 nm. The cells were incubated with 10 mM D,L-lactate for 10 min at 35°C in a rotary shaker, and then 0.05 ml of 1 M potassium arsenate (pH 6.6) or 1 M potassium arsenate–100 mM potassium phosphate (pH 6.6) was added. They were further incubated under the same conditions. At the times indicated, 0.01 ml of cell suspension was put on a slide, and the swimming pattern was observed under a phase-contrast microscope. In the case of chemotactically wild-type strains (RP487, AN180, and AN120), the population of cells showing spontaneous tumbling was counted. Cells of the *cheX* mutant (RP4080) vigorously and continuously tumble in the presence of repellent (10), and this property is convenient for the observation of change in the swimming pattern. $CoSO_4$ (0.1 mM) was added as a repellent before incubation with arsenate, and the population of continuous tumblers was observed. D,L-Lactate was added to prevent loss of motility. It is known that cells show good motility during incubation with arsenate when lactate is present (15).

Recovery of tumbling ability was observed as follows. After incubation with 10 mM arsenate for 1 to 2 h under the conditions described above, the indicated concentration of potassium phosphate (pH 6.6) was added. The change in swimming pattern was assayed as described above, and the reactivity of chemotactically wild-type strains to Co^{2+} was observed by the temporal assay (17). A 1-μl portion of 2 mM $CoSO_4$ was added to 0.01 ml of cells on the slide, and Co^{2+}-induced behavior was assayed.

Assay for cellular ATP level. Cellular ATP was extracted with 0.1 N HNO_3 (9). At the times indicated, 0.2 ml of cell suspension was injected into 0.2 ml of ice-cold 0.2 N HCO_3, and the solution was incubated for 15 min at 0°C. Then, 0.2 ml of ice-cold 0.18 N KOH–0.04 M $KHCO_3$ was added to neutralize the solution. Insoluble cell materials were removed by centrifugation.

The amount of ATP was assayed by the firefly luciferin-luciferase procedure (20, 22). Luciferin-luciferase was extracted from fireflies according to Hammerstedt (11), with some modifications. A 200-mg portion of dissected firefly tails (Sigma Chemical Co.) was homogenized in 5 ml of ice-cold buffer A (10 mM Tris-hydrochloride [pH 7.6], 50 mM potassium arsenate, 10 mM magnesium sulfate, 5 mM 2-mercaptoethanol) with a manual homogenizer at 0°C. The extract was transferred to a centrifugation tube. The homogenizer was washed with 5 ml of the same buffer, and the wash was combined with the extract. After incubation for 15 h at 4°C, the combined solution was centrifuged at 20,000 × *g* for 20 min at 4°C. The same volume (about 10 ml) of buffer A–50% (vol/vol) glycerol was added to the yellow supernatant and divided into 1-ml fractions. Each fraction was stored at −80°C. Under this condition, it was stored for several months without loss of any activity. For use in testing, a 1-ml fraction was diluted with 9 ml of buffer B (10 mM Tris-hydrochloride [pH 7.6], 10 mM potassium arsenate, 10 mM magnesium sulfate, 5 mM 2-mercaptoethanol). After addition of 0.2 ml of this diluted extract to 1.7 ml of buffer C (50 mM glycylglycine [pH 7.4], 10 mM potassium arsenate) in a vial, the background luminescence was measured. Then, 0.1 ml of sample was added to the vial and the amount of ATP was assayed. Luminescence was counted with a Packard Tri-Carb liquid scintillation spectrometer (LSC 3330, single channel; window, 2–100; gain, 31%; and counting time, 5 s from 15 to 20 s after addition). ATP level was expressed in nanomoles per milligram of protein. Protein was determined by the method of Lowry et al. (16), with bovine serum albumin as the standard.

RESULTS

Effect of arsenate on tumbling ability. The effect of arsenate on the cellular ATP level and tumbling ability of the *cheX* mutant (RP4080) was studied (Fig. 1). The ATP level of cells incubated with 10 mM D,L-lactate and 0.1 mM $CoSO_4$ was about 5.4 nmol/mg of protein. Under this condition, all cells continuously and vigorously tumbled. Addition of 10 mM arsenate caused decreases in both the cellular ATP level and the population of tumbling cells. At 15 min,

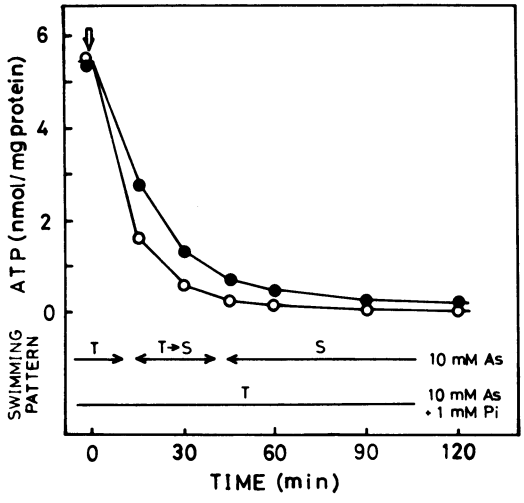

FIG. 1. *Effect of 10 mM arsenate (As) on cellular ATP level and swimming pattern of RP4080. Cells were incubated with 10 mM arsenate in the absence or presence of 1 mM phosphate. At the times indicated, cellular ATP and swimming pattern were assayed as described in the text. Symbols: ATP levels of cells incubated* (○) *with 10 mM arsenate alone and* (●) *with 10 mM arsenate–1 mM phosphate. When populations of continuous tumblers were >90% and <10%, these swimming patterns are expressed by T and S, respectively. When 10 to 90% of cells continuously tumbled and an increasing population of smooth swimmers was observed during incubation, T → S was used.*

the ATP level was about 1.5 nmol/mg of protein, and some smooth swimmers were observed. At 30 min, the ATP level was about 0.6 nmol/mg of protein, and about half of the cells showed smooth swimming. After 45 min of incubation, most of the cells swam smoothly, and the ATP level was reduced to 0.3 nmol/mg of protein. In contrast, a decrease in the ATP level was not accompanied by loss of tumbling when the cells were incubated with 10 mM arsenate–1 mM phosphate. After 2 h of incubation, the ATP level was reduced to 0.25 nmol/mg of protein. However, the cells still showed continuous tumbling under this condition. A similar result was obtained when RP487 (chemotactically wild-type strain) was studied. Cells were unable to tumble after 30 min of incubation with 10 mM arsenate. However, cells incubated with 10 mM arsenate–1 mM phosphate still tumbled, although the ATP level was reduced to a level similar to that of cells incubated with 10 mM arsenate (data not shown).

Restoration of tumbling ability by phosphate. The mutant cells swam smoothly and the ATP level was reduced to about 0.1 nmol/mg of protein after 2 h of incubation with 10 mM arsenate. The swimming pattern was not affected by 0.3 mM phosphate, whereas addition of 0.6 or 1.0 mM phosphate caused a complete restoration of tumbling. The corresponding ATP levels of the tumbling cells were as low as 0.2 and 0.3 nmol/mg of protein, respectively (Fig. 2). These observations suggest that the loss of tumbling ability in arsenate-treated cells is not due to a decrease in cellular ATP level.

To investigate the possibility described above, the relationship between cellular ATP level and tumbling ability was studied further, using the Ca^{2+},Mg^{2+}-ATPase-defective mutant AN120 (*uncA*). The mutant cells suspended in the culture medium contained about 4 nmol of ATP per mg of protein. After three washes with 10 mM PIPES buffer, the ATP level was reduced to 0.3 nmol/mg of protein. Addition of 10 mM lactate caused an increase in ATP level to 2.5 nmol/mg of protein. During these treatments, no significant change in swimming behavior was observed (data not shown). After 1 h of incubation with 10 mM arsenate, the ATP level was decreased to 0.03 nmol/mg of protein, and the cells became unable to tumble (they neither spontaneously tumbled nor responded to repellent). The tumbling ability of these cells was restored after addition of 2 or 5 mM phosphate, although the increase in ATP level was slight (to 0.03 and 0.07 nmol/mg of protein, respectively, after 20 min of incubation). More than

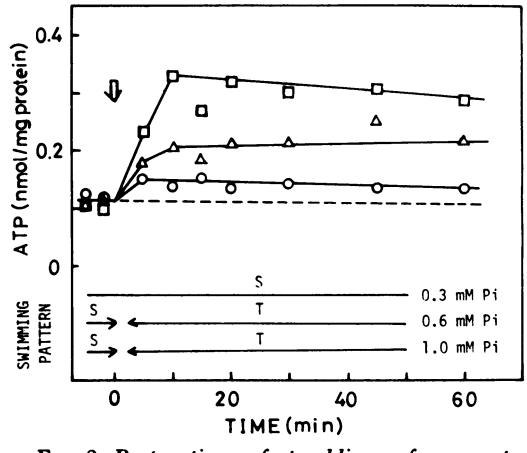

FIG. 2. *Restoration of tumbling of arsenate-treated cells by P_i. RP4080 cells were treated with 10 mM arsenate for 2 h in the presence of 10 mM* D,L*-lactate and 0.1 mM $CoSO_4$. Then, P_i was added to this cell suspension, and the swimming pattern and ATP level were assayed. Symbols: ATP level after addition of* (○) *0.3,* (△) *0.6, and* (□) *1.0 mM phosphate, respectively. S and T are used to express the swimming patterns given in the legend to Fig. 1. The dotted line shows the ATP level without the addition of phosphate.*

half of the cells showed spontaneous tumbling and about half responded to 0.2 mM Co^{2+}. After 10 min of incubation with 10 mM phosphate, the ATP level was increased to 0.1 nmol/mg of protein, and tumbling ability was almost completely restored. The cellular ATP level of AN180 (Ca^{2+},Mg^{2+}-ATPase-positive strain) was reduced to 0.16 to 0.2 nmol/mg of protein, and these cells almost completely lost tumbling ability. The enhancement of the ATP level by phosphate was much more effective than in the mutant. However, the difference observed between recovery of tumbling ability in the two strains was not particularly significant (Fig. 3, Table 1).

Restoration of tumbling in arsenate-treated *cheX* cells by phosphate addition was also observed even when cells were incubated with an inhibitor of ATPase, such as dicyclohexylcarbodiimide. However, a significant loss of motility was observed after dicyclohexylcarbodiimide treatment even in the presence of lactate (data not shown).

DISCUSSION

Arsenate treatment caused decreases in both cellular ATP level and tumbling frequency. The cells almost completely lost their tumbling ability and the ATP level was decreased to less than 0.3 nmol/mg of protein after 1 h of incubation with 10 mM arsenate alone. In contrast, the reduction in the ATP level was not accompanied by a loss of tumbling when the cells were incubated with 10 mM arsenate and 1 mM phosphate (Fig. 1). The lack of correlation between tumbling ability and cellular ATP level was also observed in the following cases. (i) The tumbling ability of the arsenate-treated cells was restored by addition of phosphate, even when the ATP level was as low as 0.2 nmol/mg of protein (Fig. 2). (ii) Addition of phosphate caused only a slight increase in ATP level of the arsenate-treated mutant AN120 (*uncA* mutant). Tumbling ability was almost completely restored by addition of 5 mM phosphate, although the ATP level was only 0.07 nmol/mg of protein (Fig. 3, Table 1). These findings suggest that the lack of tumbling ability in arsenate-treated cells is due to the decrease in the level of some phosphorylated compound other than ATP and that the cellular level might be influenced not by the ATP level but by the ratio of arsenate to phosphate in the cell. In other words, this paper suggests an involvement of an unidentified factor, neither ATP nor an ATP derivative such as *S*-adenosylmethionine, in tumbling. Since inorganic phosphate is involved in a wide variety of cellular reactions, further studies would be required for the identification of the compound.

The results presented in this paper show that the cellular ATP level is independent of the lack of tumbling in arsenate-treated cells. However, nothing can be said about any ATP requirement for tumbling. There is a possibility that arsenate-treated cells still contain more than the threshold concentration of ATP required for tumbling. Therefore, the possibility that ATP plays some

TABLE 1. *Effect of P_i on swimming pattern of arsenate-treated cells*[a]

P_i (mM)	Swimming pattern			
	Nonstimulated condition		Presence of Co^{2+}	
	AN180	AN120	AN180	AN120
0	S	S	S	S
2	W	W ~ S	T > S	T < S
5	W	W > S	T	T > S
10	W	W	T	T
20	W	W	T	T

[a] Arsenate-treated cells were incubated with the indicated concentration of P_i for 10 min. Their swimming patterns under nonstimulated conditions and their response to 0.2 mM $CoSO_4$ were observed as described in the text. Cellular ATP levels of these cells are shown in Fig. 4. S, W, and T indicate smooth, wild-type, and tumbling swimming, respectively. When the predominant patterns were smooth swimming and wild-type (or tumbling) swimming, these patterns are expressed as T < S and W > S (or T > S), respectively. W ~ S indicates roughly half in each swimming pattern.

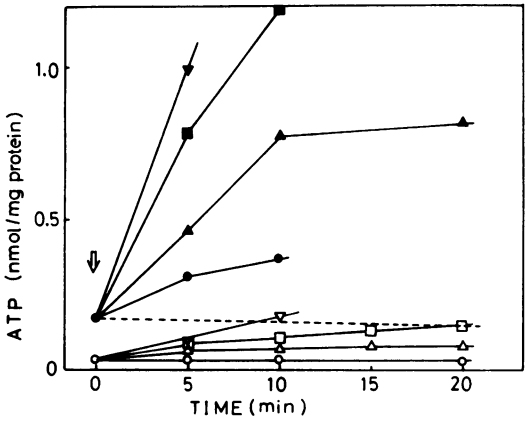

FIG. 3. *Effect of P_i on ATP level of arsenate-treated cells. Cells of AN180 (uncA$^+$) and AN120 (uncA) were treated with 10 mM arsenate for 1 h, and then P_i was added. At the times indicated, cellular ATP levels of these cells were measured. Swimming patterns are shown in Table 1. Open and closed symbols indicate AN120 and AN180, respectively: cellular ATP levels after addition of* (○, ●) *2,* (△, ▲) *5,* (□, ■) *10, and* (▽, ▼) *20 mM phosphate, respectively.*

role in tumbling cannot be eliminated. Table 1 shows that an *uncA* mutant requires more phosphate for recovery of tumbling than an *uncA*$^+$ strain does. Since the ATP level of AN120 after arsenate treatment was very low (only 10 to 20% of that of the *uncA*$^+$ strain), it is possible that the ATP level of arsenate-treated AN120 was around the threshold concentration whereas that of the *uncA*$^+$ strain was above it.

Since scarcely anything is known about sensory excitation, it is difficult at present to speculate about the cause and mechanism of elimination of tumbling ability by arsenate treatment. However, that arsenate-treated cells cannot tumble is an important fact for the study of sensory excitation.

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