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Histidine Starvation and Adenosine 5'-Triphosphate Depletion in Chemotaxis of *Salmonella typhimurium*

RICHARD J. GALLOWAY AND BARRY L. TAYLOR*

Department of Biochemistry, School of Medicine, Loma Linda University, Loma Linda, California 92350

Starvation for histidine prevented tumbling in *Salmonella typhimurium hisF* auxotrophs, including constantly tumbling strains with an additional mutation in *cheB* or *cheZ*. However, histidine-starved $cheZ_S$ *hisF* strains were not defective in flagellar function or the tumbling mechanism since freshly starved auxotrophs tumbled in response to a variety of repellents. Tumbling in histidine-starved *S. typhimurium* could be restored in 13 s by addition of adenine or in 4 min by addition of histidine. Chloramphenicol did not prevent restoration of tumbling by these substances. Assays of adenosine 5'-triphosphate were performed based upon previous demonstration of adenine depletion in *hisF* auxotrophs starved for histidine. The adenosine 5'-triphosphate concentration dropped rapidly during the course of starvation, falling to less than 5% of the initial level as the cells ceased tumbling entirely. The change to smooth motility was prevented by 2-thiazolealanine, which inhibits phosphoribosyltransferase, thereby preventing adenine depletion during histidine starvation. These results suggest that an adenosine 5'-triphosphate deficiency was responsible for the change in tumbling frequency.

Bacterial chemotaxis involves an elementary chemosensory system which is a useful model for neurosensory systems in more complex organisms (13). The normal random swimming pattern of peritrichous bacteria consists of straight swimming (runs) interrupted occasionally by tumbles in which the cells abruptly change direction. The bacteria are able to respond to gradients of attractants and repellents in their environment by regulating the frequency of tumbling so that their net movement is biased toward the most favorable environment (6, 20).

Combined use of genetic and biochemical techniques has begun to yield a fine dissection of the molecular mechanism of chemotaxis (9, 14, 19, 31, 37), but the challenge to discover the biochemical role of most of the products specified by chemotaxis genes remains. Although nine genes are known to be essential for chemotaxis in *Salmonella typhimurium* (5, 7, 40), the reactions catalyzed by the gene products have been identified for the $cheR_S$ and $cheB_S$ genes only (33, 35; an explanation of the gene nomenclature is found in Materials and Methods). The $cheR_S$ product is a methyl transferase which methylates membrane-bound, methyl-accepting chemotaxis proteins by using *S*-adenosylmethionine as the methyl donor (33). The extent of methylation is proportional to the fraction of chemotaxis receptors that are occupied by attractant ligands (8). The $cheB_S$ gene codes for a methyl esterase that hydrolyzes the methyl ester formed by the methyl transferase (35).

The discovery of the role of methylation in chemotaxis was the end result of the observation that methionine auxotrophs of *Escherichia coli* become nonchemotactic when starved for methionine (1). The bacteria swim smoothly without tumbling and are therefore unable to bias their swimming in a favorable direction. Subsequent studies have shown that it is the depletion of *S*-adenosylmethionine in methionine starvation that is responsible for the loss of chemotaxis (3, 4). *S*-adenosylmethionine is the methyl donor for the methylation of the methyl-accepting chemotaxis proteins, and methylation is essential for adaptation to stimuli. Methionine-starved bacteria swim smoothly because of a deficiency in adaptation to stimuli rather than a defective tumble-generating mechanism (4, 30).

In the process of developing methods for decreasing the steady-state tumbling frequency in a constantly tumbling strain of *S. typhimurium*, ST171, we discovered that histidine starvation also caused smooth motility. The histidine effects on chemotaxis appeared to be mediated by ATP. This discovery has the potential to provide new insights into the molecular mechanism of chemotaxis.

(A report of this work was presented at the XIth International Congress of Biochemistry in Toronto, July 1979.)

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of *S. typhimurium* used in this study are described in Table 1. Except where stated otherwise, cells were grown aerobically at 30°C in Vogel and Bonner (39) salts medium with citrate, fortified with the auxotrophic requirements of the strain. Glycerol (1.0%, vol/vol) or glucose (0.7%, wt/vol) was added as the carbon source. The inoculum for starvation studies was prepared by growth to mid-exponential phase in nutrient broth and stored at 5°C for up to 1 month.

Observation of motility and chemotactic responses. A small drop (10 μl) of bacterial culture was placed on a microscope slide, and the bacteria were observed at a magnification of ×500 through a Leitz Dialux trinocular microscope with dark-field optics and an objective lens with a long working distance (UMK 50 or L32). Behavioral responses were examined by using a temporal assay procedure (20) in which 1 μl of a 10× concentrated solution of attractant or repellent was mixed with a 10-μl drop of culture on the microscope slide. The swimming pattern of the bacteria was observed before and after the stimulus. To facilitate analysis of the behavior the microscope was fitted with interchangeable photographic (Nikkormat ELW) and video (camera, Sanyo Model VC 3300X; recorder, Sanyo model VTC 7100; monitor, Hitachi Model VM-172) equipment.

For observation of the light response, bacteria were illuminated with a high-intensity lamp (Osram HBO 100W/2) as described previously (36, 37). For normal observation, a long pass orange filter (Oriel 5150; 50% transmission, 530 nm) was inserted in the light path to protect bacteria from the effect of intense blue light (21). The effect of a pulse of blue light on cell motility was observed when the filter was briefly removed from the light path.

Measurement of swimming velocities. A modification of the photographic procedure of Spudich and Koshland (34) was used to measure velocities. A 2-s exposure was made of bacteria illuminated with 5-Hz stroboscopic illumination. The photographic negative was projected, and migration of bacteria during four flashes of light was measured with a Panasonic electronic ruler (model 8210). Distances were calibrated by projecting a micrometer scale photographed through the microscope.

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
ST23	*hisF8786 thyA1981*	D. E. Koshland, Jr.
ST171	*hisF8786 thyA1981 cheZ221*	Aswad and Koshland (5)
TT218	*metE862*::Tn*10*	J. Roth
TT21	*serB965*::Tn*10*	J. Roth
TT191	*thr-557*::Tn*10*	J. Roth
BT10	*hisF8786 thyA1981 cheZ221 metE862*::Tn*10*	TT218 × ST171[a]
BT15	*hisF8786 thyA1981 cheZ221 thr-557*::Tn*10*	TT191 × ST171
BT13	*hisF8786 thyA1981 cheZ221 serB965*::Tn*10*	TT21 × ST171
BT14	*hisF8786 thyA1981 thr-557*::Tn*10*	TT191 × ST23
SL4041	*trpA8 hisC527*(Am) *cheB111*(Am)	Vary and Stocker (38)
ST313	*cheB111*::Tn*10*	D. E. Koshland, Jr.
BT11	*hisF8786 thyA1981 cheB111*::Tn*10*	ST313 × ST23
ST383	*hisF8786 thyA1981 met*	D. E. Koshland, Jr.
ST384	*hisF8786 thyA1981 leu*	D. E. Koshland, Jr.
ST388	*hisF8786 thyA1981 ilv*::Tn*10*	D. E. Koshland, Jr.

[a] Strains were constructed by transduction with P22 (*int3*).

Measurement of ATP. ATP was extracted with trichloroacetic acid by the procedure of Lundin and Thore (17, 18). The concentration of ATP in the extract was assayed with the LKB-Wallac ATP monitoring kit, which uses a purified luciferase-luciferin reagent.

Nomenclature. The nomenclature of the *che* genes in *S. typhimurium* and *E. coli* evolved independently; as a result, different designations were given to homologous genes in the two species (7). For clarity we have followed the suggestions of Parkinson and Koshland (personal communication) and used a common symbol for homologous genes in *S. typhimurium* and *E. coli*. A subscript E is used to indicate *E. coli* genes, and a subscript S is used to indicate *S. typhimurium* genes. The new and old nomenclatures are shown in Fig. 1.

RESULTS

Methionine starvation. Methyl esterase-deficient (*cheB*) strains of *E. coli* and *S. typhimurium* have a constantly tumbling phenotype in which the bacteria perform an erratic turning motion without any runs (5, 26). Methionine starvation is less effective in decreasing the tumbling frequency of *cheB* strains than it is in decreasing the tumbling frequency of *che*$^+$ strains (3). No change in tumbling frequency is observed when *S. typhimurium* ST4 (*cheB*$_S$ *111*) and one of the *cheB* strains of *E. coli* are starved for methionine (3, 4, 32). The *cheZ* strains of *S. typhimurium* and *E. coli* comprise another class of mutants that have a constantly tumbling phenotype (5, 26, 40). It is possible that the tumble-generating system of ST171 (*cheZ*$_S$) would be more susceptible to loss of methionine than that of esterase-deficient strains.

To investigate this possibility we transduced into ST171 the *metE862*::Tn*10* (Tet^r) locus from TT218. The transductant, designated BT10, was resistant to tetracycline and required methionine for growth. Starvation for methionine was

Original Nomenclature	S. typhimurium	P	Q	R	S	T	U	V	W	X
	E. coli	A	Y	X	–	Z	C	–	W	B
New Nomenclature	Subscripts S and E to indicate species	A	Y	R	S	Z	C	V	W	B

FIG. 1. *Comparison of new designations for homologous che genes in E. coli and S. typhimurium with the old usage.*

achieved by washing the bacteria three times in methionine-free glucose medium with histidine and thymine supplements and suspending the pellet in the same medium. There was a marked reduction in the tumbling frequency of BT10 when the cells were deprived of methionine (Fig. 2). Although there was some variation from day to day, the motility of methionine-starved BT10 was usually random. Cycloleucine (50 mM), which inhibits synthesis of *S*-adenosylmethionine (16), did not appear to decrease the tumbling frequency in methionine-starved or unstarved BT10. It was subsequently determined that starvation for histidine was more effective than starvation for methionine in abolishing tumbling in both BT10 and in BT11 (*hisF thyA cheB*$_S$ 111).

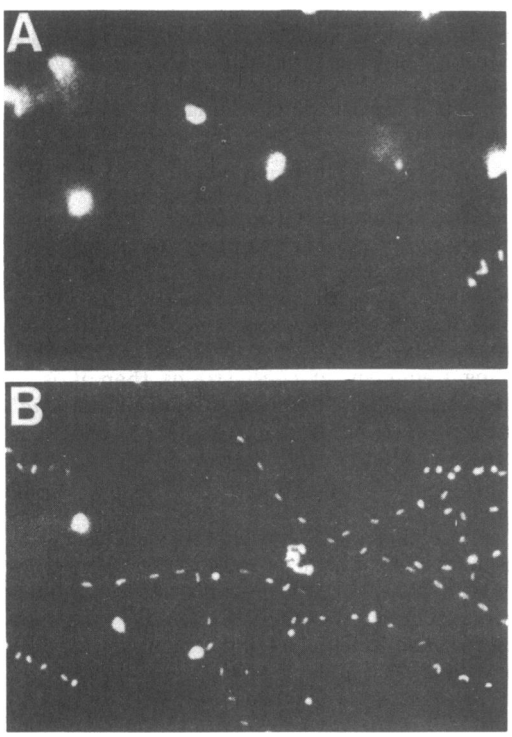

FIG. 2. *Motility pattern of S. typhimurium BT10 in the presence and absence of methionine. BT10 was grown to exponential phase (E_{600}, 0.5) in Vogel-Bonner medium with citrate supplemented with glucose (37 mM), thymine (200 μM), histidine (160 μM), and methionine (150 μM). The cells were harvested, washed three times in methionine-free medium, suspended in medium with or without methionine, and incubated at 30°C for 3 h. Motility was photographed using stroboscopic illumination (5 Hz) and a 2-s exposure. A, In methionine medium constantly tumbling BT10 were recorded as splotches. B, In methionine-free medium the random motility appeared as tracks.*

Histidine starvation. BT10 has auxotrophic requirements for histidine and thymine in addition to methionine. When BT10 in glucose medium supplemented with methionine and thymine was starved for histidine by a procedure similar to the methionine starvation, the cells became smooth swimming 2.5 to 3.0 h after the start of washing (Fig. 3). Typically, the constantly tumbling motility was unchanged during the first 2.5 h and then in the next 0.5 h rapidly changed to smooth swimming with a very low tumbling frequency (less than 0.2 tumble per s). The transition to smooth swimming proceeded through a random swimming phase in which the bacteria tumbled approximately once per second. Similar results were obtained when BT11 was starved for histidine. Histidine-starved BT10 in glucose medium continued to swim smoothly for about 18 to 20 h, at which time the motility became random. Motility remained vigorous throughout the period of observation, so histidine-starved BT10 is well suited

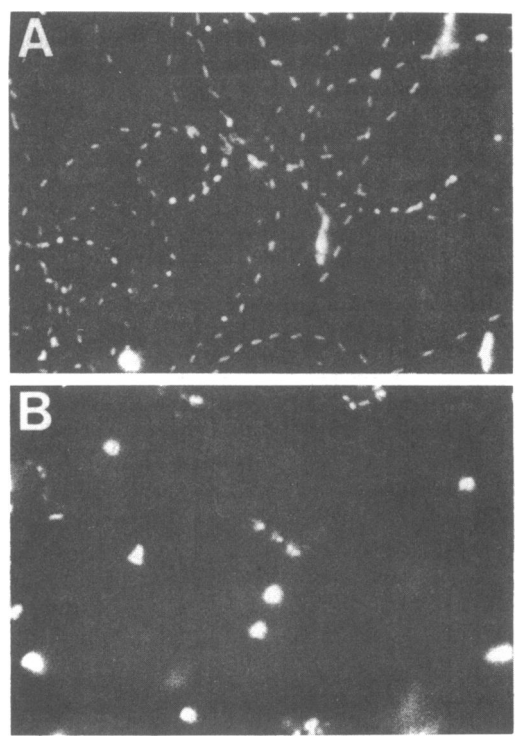

FIG. 3. *Motility pattern of histidine-starved S. typhimurium in the presence and absence of adenine. Cells were prepared as described in the legend to Fig. 2, except that histidine was omitted from the medium instead of methionine. A, Smooth motility pattern of histidine-starved BT10. B, Constantly tumbling motility photographed 1 min after the addition of adenine (1.5 mM) to the histidine-starved cells in A.*

to the study of negative (tumbling) responses in *cheZ*$_S$ strains. BT10 starved for histidine in glycerol medium adopted a random motility pattern about 2 h after becoming smooth. Histidine starvation also caused ST23 (*che*$^+$ *hisF thyA*) to become smooth swimming.

The starvation effect appears to be specific for histidine and methionine and is not due to stringent control. The strains listed in Table 2 were derived by transducing an additional auxotrophic requirement into ST23 or ST171. The transductants were screened for starvation effects. Histidine starvation caused smooth swimming in all of the strains tested. Methionine starvation eliminated tumbling in a *che*$^+$ strain (ST383), in agreement with the findings of Aswad and Koshland (3). Starvation for amino acids other than histidine and methionine did not significantly change tumbling frequency.

It is possible that smooth swimming in histidine-starved bacteria was the result of an impairment of motility. The source of energy for motility in bacteria is the proton motive force across the cytoplasmic membrane (15, 22). If the proton motive force falls below a critical level, the tumbling frequency in *S. typhimurium* is sharply reduced. A continued decrease in the proton motive force causes smooth swimming and, eventually, paralysis when the proton motive force drops below the critical level for motility (11; D. J. Laszlo and B. L. Taylor, submitted for publication). If decreased proton motive force were the cause of smooth swimming, the speed of swimming would usually be slower than normal, because the flagellar motors are not operating at maximum capacity. The speed of histidine-starved BT10 was measured in glucose and glycerol medium and found to be similar to the speed of BT10 in histidine medium (Table 3). This suggests that the smooth swimming did not result from a low proton motive force in the histidine-depleted bacteria. Furthermore, histidine-starved ST171 and BT10 are not defective in the tumble-generating mechanism. A temporal gradient of indole (0 to 0.4 mM) caused a prolonged tumbling response in freshly starved cells.

TABLE 2. *Motility of S. typhimurium after starvation for an auxotrophic requirement*[a]

Strain	Genotype	Requirement withheld	Motility
ST23	*hisF thyA*	None	Random
		Histidine	Smooth
		Thymine	Random
ST388	*hisF thyA ilv*	Isoleucine, valine	Random
ST384	*hisF thyA leu*	Leucine	Random
BT14	*hisF thyA thr*	Threonine	Random
ST383	*hisF thyA met*	Methionine	Smooth
BT10	*hisF thyA metE*::Tn*10* *cheZ*	None	Tumbling
		Histidine	Smooth
		Thymine	Tumbling
		Methionine	Random
BT11	*hisF thyA cheB*::Tn*10*	None	Tumbling
		Histidine	Smooth
BT15	*hisF thyA thr cheZ*	Threonine	Tumbling
BT13	*hisF thyA serB cheZ*	Serine	Tumbling

[a] Three drops of a nutrient broth culture were used to inoculate 5 ml of Vogel-Bonner citrate medium supplemented with glucose and the auxotrophic requirements of the strain other than the requirement for which the strain was being starved. The strains were incubated at 30°C for 15 h, and motility was observed in the microscope.

Evidence for adenine depletion. Reversal of the smooth swimming caused by histidine starvation was studied in bacteria that had been depleted of histidine and were smooth swimming for 0.5 to 2.0 h (Table 4). Histidine (1.0 mM) restored constantly tumbling motility to starved ST171. Even though histidine starvation has some of the same characteristics as methionine starvation, concentrations of methionine as high as 25 mM did not increase tumbling frequency. We screened thirty-two additional amino acids and vitamins in a search for other nutrients that could reverse the loss of tumbling in histidine starvation. Only adenine fully restored tumbling, although guanine was partially effective (Table 4). The mean time required to restore constant tumbling to histidine-starved ST171 was 13 s for adenine and 4.2 min for histidine. The addition of adenine to growth medium without histidine

TABLE 3. *Speed of BT10 before and after histidine starvation*[a]

Incubation medium	Velocity ($\mu m\ s^{-1}$)
Glycerol with histidine	22.8 ± 0.7
Glycerol without histidine	23.6 ± 1.4
Glucose with histidine	28.4 ± 0.6
Glucose without histidine	27.5 ± 1.4

[a] BT10 was incubated for 15 h at 30°C in Vogel-Bonner citrate medium with glucose (0.7%) or glycerol (1%) as the carbon source. The medium was supplemented with thymine (0.13 mM) and methionine (0.16 mM) as well as histidine (0.14 mM) where indicated. The cells were diluted in the same medium to approximately 5×10^7 cells per ml. The motility pattern was smooth for histidine-starved cells and tumbling for unstarved cells. Serine (0.1 mM) was added, and the speed was measured immediately by the photographic procedure (see text). The speeds given are the mean of 10 measurements ± standard error of the mean.

TABLE 4. *Effect of added nutrients on the motility of histidine-starved ST171*[a]

Addition (150 μg/ml)	Motility
None	Smooth
Histidine	Tumbling
Methionine	Smooth
Adenine	Tumbling
Guanine	Some tumbling
Thymine	Smooth
Cytosine	Smooth

[a] ST171 was depleted of histidine by washing twice and resuspending the cells in Vogel-Bonner medium (5 ml) with glucose and thymine. The motility pattern became smooth in 2.5 h. After ST171 were smooth swimming for 0.5 h to 2.0 h, the indicated nutrient was added to the medium, and motility was determined after an additional 4 h of incubation at 30°C with shaking. The concentration of additives was 2 mg per ml, except that vitamins were 0.2 mg per ml. Other nutrients tested and found to be ineffective in restoring tumbling were: alanine, *p*-aminobenzoic acid, arginine, asparagine, aspartate, biotin, cysteine, diaminopimelic acid, glutamate, glutamine, glutathione, glycine, *p*-hydroxybenzoic acid, isoleucine, leucine, lysine, nicotinic acid, pantothenate, phenylalanine, proline, pyridoxal, serine, thiamine, threonine, tryptophan, tyrosine, uracil, and valine.

prevented the loss of tumbling in ST171, but did not satisfy the growth requirement for histidine. No significant growth was detected when ST171 was incubated in this medium for 24 h.

Protein synthesis is not required for the restoration of tumbling by histidine or adenine, because the presence of chloramphenicol (100 μg/ml) did not prevent restoration of tumbling by those compounds. It is noteworthy that chloramphenicol alone restored tumbling in 40 min and, when present during starvation, prevented a loss of tumbling. Presumably, this is because starvation for an essential amino acid stimulates proteolysis and thereby increases the pool of endogenous histidine (25, 27). Simultaneous starvation of BT10 for both methionine and histidine also failed to yield loss of tumbling.

The effectiveness of adenine in restoring tumbling suggests that it is the loss of an adenine compound and not the loss of histidine per se which is responsible for the loss of tumbling. Because ATP is required for the synthesis of *S*-adenosylmethionine and may be required per se for chemotaxis, we measured the intracellular concentration of ATP in BT10 before and during histidine starvation. The results (Table 5) confirm that the intracellular concentration of ATP is sharply decreased by starvation for histidine.

2-Thiazolealanine is an analog of histidine that blocks histidine biosynthesis (and ATP consumption) by binding at the regulatory site of the *hisG* enzyme, phosphoribosyl-ATP synthetase (2). In a wild-type cell the *hisG* enzyme, the first in the pathway for histidine biosynthesis, is regulated by feedback inhibition by histidine. Thiazolalanine (20 μg/ml) protects ST171 against changes in tumbling frequency during 6.5 h of histidine starvation. The addition of thiazolealanine (50 μg/ml) to histidine-starved ST171 also restored tumbling in 30 min.

TABLE 5. *Effect of histidine starvation on the intracellular ATP pool in BT10*[a]

Time (h)	Motility	ATP (mM)
0	Constantly tumbling	3.6
1.75	Mostly tumbling	0.55
2.75	Smooth	0.16
4.5	Smooth	0.06

[a] BT10 was grown in Vogel-Bonner glucose medium as described in Table 3. The 15-h culture (absorption at 620 nm, 0.42) was harvested by centrifugation, washed twice, and suspended in the same medium minus histidine.

Response of ST171 to chemotactic stimuli. The *cheZ*$_S$ mutation in ST171 does not block the chemotactic response to favorable stimuli such as a temporal increase in attractant (5, 34). We have confirmed this finding, but preliminary studies of strains with a series of independent *cheZ*$_S$ mutations indicate that the length of the smooth-swimming response to serine (0 to 10 μM) is 5 to 20% of the response time of ST23 (*che*$^+$) to the same temporal gradient (R. D. Jaecks and B. L. Taylor, unpublished data). The response of the *cheZ*$_S$ strains to aspartate (0 to 10 μM) is affected less severely and ranges from 49 to 92% of the response by ST23. This is similar to the results obtained for *E. coli cheZ*$_E$ strains: the serine response is impaired to a greater extent than the aspartate response (7).

The responses of histidine-starved *S. typhimurium* to the attractants serine and aspartate were examined by taking advantage of the additivity of responses to blue light and attractants. A pulse of intense blue light causes *S. typhimurium* to tumble briefly (21, 36). During the smooth (positive) swimming response to an attractant the bacteria are protected against the (negative) stimulus of blue light. As a result, the length of the smooth response to an attractant can be measured in histidine-starved smooth-swimming bacteria by timing the interval in which the culture is protected against blue light. The responses of histidine-starved ST23 to serine and aspartate measured in this manner were similar to the responses of unstarved bacteria measured by the same procedure, suggesting that the starved bacteria are not deficient in adaptation to an attractant stimulus.

ST171 and BT10 have been observed to re-

spond to a variety of repellents, including phenol, benzoate, and indole. The results obtained were complex, but it is possible to make some generalizations. As starvation proceeds, *che*$^+$ and *cheZ*$_S$ strains progressively lose the response to repellents (Table 6). Indole (0.4 mM) remained effective for a time after other repellents failed to induce a response (Table 6). Starvation was more effective when the carbon source was glucose rather than glycerol. This is indicated (Table 6) by the absence of any response to indole in 18-h glucose-grown cells, whereas 18-h glycerol-grown cells consistently responded to indole.

The progressive changes in repellent responses appear to be due to histidine starvation, because they are qualitatively similar in *che*$^+$ and *cheZ*$_S$ strains. However, specific differences have been observed in *cheZ*$_S$ strains. In ST23 freshly starved for histidine in glycerol or glucose medium, the mean response time to a temporal gradient of indole (0 to 0.4 mM) was about 1 min. The response of freshly starved ST171 or BT10 to the same gradient of indole lasted at least 6.5 min and was sometimes much longer. Likewise, with other repellents the response of *cheZ*$_S$ strains lasted much longer than the response of *che*$^+$ strains.

DISCUSSION

A procedure has been developed that permits the study of negative (tumbling) chemotactic responses in mutants which normally have a constantly tumbling phenotype. Starvation for histidine reduced the frequency of tumbling in BT10 to near zero (Fig. 3), but motility was otherwise unaffected, and the bacteria continued to swim vigorously for up to 36 h. Histidine starvation progressed through several stages. Tumbling first became occasional (random motility) and then essentially ceased (smooth motility). Even after spontaneous tumbling had ceased the bacteria were able to tumble in response to repellents and light. As starvation continued the cells became unresponsive to blue light (weaker stimulus) and eventually to repellents (stronger stimulus). The protection against negative stimuli that was afforded by prolonged histidine starvation is consistent with the observation by Springer and Koshland (33) that histidine starvation blocks the response to repellents in *S. typhimurium* ST1038 (*cheR*$_S$ *hisF thyA*). The recovery of spontaneous tumbling was observed in ST171 that had been starved for histidine for an extended interval (Table 6). This occurred more rapidly when the carbon source was glycerol rather than glucose. The recovery of spontaneous tumbling was not accompanied by a recovery of lost repellent responses and was therefore not a reversal of histidine starvation.

TABLE 6. *The effect of extended histidine starvation on the response of ST171 to repellents*[a]

Carbon source	Time of starvation (h)	Repellent[b]	Motility
Glycerol	2.5	None	Smooth
	5.5	None	Random
		1 mM Phenol	Tumbling
	6.5	1 mM Phenol	Random
	18	0.4 mM Indole	Tumbling
Glucose	2.5	None	Smooth
	18	None	Smooth
		0.4 mM Indole	Smooth
	22	None	Random

[a] Starvation was initiated by inoculating 3 drops of a nutrient broth culture into 5 ml of Vogel-Bonner medium supplemented with the indicated carbon source and auxotrophic requirements, except histidine. Incubation was at 30°C in a gyratory shaker. Repellent effects were examined by transferring 10 μl of the incubating bacteria to a microscope slide. After observation of the motility, the bacteria on the microscope slide were rapidly mixed with 1 μl of a 10× concentrated solution of the repellent. The motility recorded was that observed 10 s after mixing.

[b] Final concentration after mixing with the bacterial culture.

Of the auxotrophic requirements tested only histidine was effective in causing smooth swimming in BT10 (Table 2). Starvation for methionine greatly decreased the tumbling frequency in BT10, but did not eliminate tumbling (Table 2). In cells starved for histidine, suppression of tumbling was not caused by depletion of histidine itself, but resulted from a secondary depletion of an adenine compound or closely related metabolite. Exogenous adenine protected against the loss of motility during histidine starvation. Adenine also restored tumbling more rapidly than did histidine when added to BT10 starved for histidine in adenine-free medium.

It has previously been shown that in histidine-starved *E. coli*, adenine is depleted by unrestrained consumption of ATP (28, 29). The first step in histidine biosynthesis (Fig. 4) is the use of ATP to form phosphoribosyl-ATP. This step is regulated primarily by feedback inhibition of phosphoribosyltransferase by histidine (23). In the absence of exogenous histidine, a defective *hisF* enzyme not only releases phosphoribosyltransferase from feedback inhibition but also prevents the synthesis of 4-amino-5-imidazole carboxamide ribonucleotide, which is required for resynthesis of ATP (Fig. 4). The result is a rapid depletion of intracellular ATP and excretion of accumulated substrate for the *hisF* en-

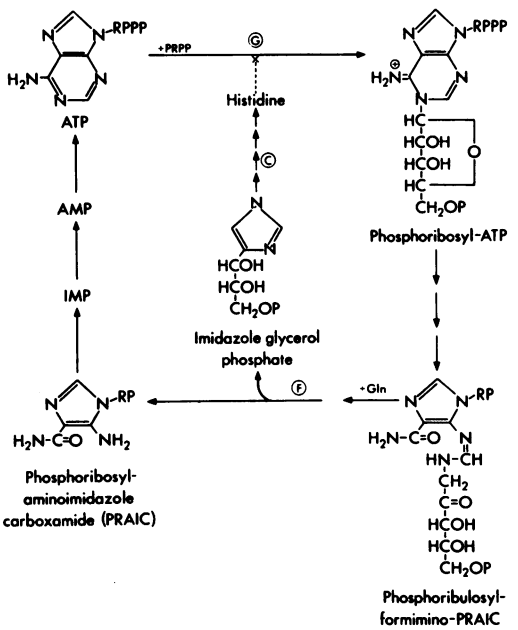

FIG. 4. *The purine nucleotide cycle in histidine biosynthesis. The products of the hisC, hisF, and hisG genes are designated by C, F, and G, respectively. Histidine inhibition of G is indicated by the broken line.*

zyme. The histidine analog 2-thiazolealanine is a potent inhibitor of phosphoribosyltransferase and is therefore able to prevent adenine depletion in histidine starvation (2, 29). It appears that a similar mechanism in *S. typhimurium* is responsible for the observed changes in the motility of BT10 as a result of histidine starvation. The starvation-induced change in motility was accompanied by a marked depletion of intracellular ATP (Table 5). The change in motility was prevented by thiazolealanine. Phenotypic adenine auxotrophy also occurred in mutants of *S. typhimurium* in which phosphoribosyltransferase is derepressed or resistant to feedback inhibition or both (10, 24). We would predict that these mutants would also have a decreased tumbling frequency. Aswad and Koshland (3) previously reported that histidine starvation of *S. typhimurium* ST42 (*cheZ*$_S$ *hisC trpA*) and ST4 (*cheB*$_S$ *hisC trpA*) did not affect tumbling frequency or chemotaxis. This observation, which we have confirmed, is not surprising. The reaction catalyzed by the *hisC* enzyme is after the formation of 4-amino-5-imidazole carboxamide ribonucleotide in the pathway for histidine synthesis. As a result, ATP consumed by phosphoribosyltransferase can be regenerated from 4-amino-5-imidazole carboxamide ribonucleotide in *hisC* mutants.

It is assumed that ATP is the adenine compound required for a normal tumbling frequency. Arsenate, which depletes the cell of ATP (12), causes smooth swimming in constantly tumbling mutants of *S. typhimurium* and *E. coli* (4, 32). The loss of ATP in histidine starvation also appears to be synchronous with changes in motility (R. Chinnock and B. L. Taylor, unpublished observation). There are three possible mechanisms by which ATP depletion could affect chemotaxis: (i) limitation of the synthesis of *S*-adenosylmethionine, thereby effecting smooth swimming by a mechanism similar to the effect of methionine starvation on tumbling (4); (ii) reduction of the proton motive force (11); or (iii) a requirement for chemotaxis of ATP per se in addition to *S*-adenosylmethionine (4, 32). At present there is insufficient evidence to distinguish between these alternatives. The change in repellent response during extended histidine starvation may reflect an effect of ATP depeletion that is different from the mechanism responsible for the initial change in tumbling frequency. The observed normal adaptation to attractants in ST23 starved for histidine suggests that *S*-adenosylmethionine depletion is not the primary cause of smooth swimming in freshly starved bacteria.

Further investigation of the mechanism of changes in tumbling frequency in histidine-starved *cheZ* strains should resolve the uncertainty about the roles of ATP in chemotaxis. It will also be possible to examine in detail the response of *cheZ*$_S$ strains to both positive and negative chemotactic stimuli. The swimming pattern of *cheZ*$_S$ strains could be a useful indicator of ATP concentration in studies of the mechanism of adenine depletion in histidine starvation.

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