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Recognition Sites for Chemotactic Repellents of *Bacillus subtilis*

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Repellents of *Bacillus subtilis* include many membrane-active compounds, such as uncouplers of oxidative phosphorylation, local anesthetics, chlorpromazine (a central nervous system depressant), and tetraphenylboron (a lipophilic anion). Normally, bacteria swim smoothly, and occasionally tumble, but addition of repellent causes all bacteria to tumble, then later resume original frequency of swimming and tumbling (adaptation). Bacteria adapted to repellent can then be tested to determine the minimum concentration (threshold) of the same or different repellents that causes tumbling. The results indicate that repellents act at (saturable) recognition sites, which differ for chemically different species. An implication is that uncouplers of oxidative phosphorylation affect cell properties by interaction at specific locations.

Peritrichously flagellated bacteria have a simple behavioral repertoire: they swim or tumble. Swimming is caused by counterclockwise rotation of flagella, tumbling by clockwise rotation (6). In tumbling, the bacteria thrash about, without making much forward progress, and the effect of tumbling is randomly to reorient the bacteria for the next swim (1). When the bacteria travel up gradients of attractant, they swim for longer times between tumbles than when in isotropic medium (1). Thus, they accumulate at regions of high concentrations of attractant, a process known as chemotaxis. The behavior underlying chemotaxis can be evoked by adding attractant to bacteria and observing that they swim smoothly for awhile, then return to their natural frequency of tumbling and swimming (adaptation) (7, 10). Conversely, repellent causes transient tumbling (10, 14).

Although the behavioral basis of chemotaxis, mentioned above, is largely understood, the biochemical events that underlie it are unknown. The two major questions are: (i) what cell parameter controls the switch that causes the flagella to rotate counterclockwise or clockwise, and (ii) how do the chemoreceptors affect this cell parameter? One approach for investigating these questions has been the study of repellents in *Bacillus subtilis*. Unlike repellents of the gram-negative bacteria, *Escherichia coli* and *Salmonella typhimurium*, which act at orthodox chemoreceptors as do attractants (14, 15), many of the repellents of *B. subtilis* are membrane-active agents. These repellents include uncouplers of oxidative phosphorylation, local anesthetics, and tetraphenylboron (TPB), a permeant anion (10, 11). Ordal and Goldman (11) have proposed that such agents act directly on the membrane to cause tumbling. Therefore, to further our understanding of chemotaxis and, in general, of the effects of these reagents on membranes, I have sought to find out whether they interact at recognition sites to cause tumbling. I conclude that they do.

MATERIALS AND METHODS

Bacteria. *B. subtilis* OI8 and OI300, derived from OI8, have been described previously (10, 11). Strain OI8 swims smoothly, but occasionally tumbles.

Microscopy assay. This assay has been described (10, 11). Basically, bacteria are grown in nutrient broth to 163 Klett units (filter 66), about 3.5×10^8 bacteria/ml, and supplemented with 5 mM sodium lactate when lactate is to be used in the suspension chemotaxis buffer. Growth is continued for 15 min. Three-tenths milliliter is filtered (Millipore filter) and washed in chemotaxis buffer (10) containing either 5 mM sodium lactate or 2 mM potassium glutamate, respectively, as an energy source. The bacteria are diluted into 3 ml and held at room temperature (21 to 23 C) in a 6-ounce (ca. 180-ml) prescription bottle, flat side down. Bacteria are transferred to a clean microscope slide as a drop, and reagent is squirted in using a disposable microcapillary. Tumbling frequency of bacteria is observed until it returns to that characteristic of untreated bacteria. To increase reliability of the assay, the reagent added is always taken from one of several tubes, at least one of which is buffer, in a blind experiment.

Chemicals. FCCP, CCP, and *p*COOH-CCP (see key to abbreviations, below) were obtained from P. G. Heytler (E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.), to whom I am grateful. Lidocaine was obtained as a gift from Astra Pharmaceutical Co., Worcester, Mass. Chlorpromazine was obtained as a gift from Smith Kline & French Laboratories, Philadelphia, Pa. All other chemicals were obtained from commercial sources. TPB was used as a sodium salt.

Respiration. Bacteria were inoculated from a stationary-phase culture in tryptone broth (1.0% tryptone, Difco; 0.5% NaCl) into nutrient broth (0.8% nutrient broth, Difco) and grown to 140 Klett units. They were centrifuged, washed twice, and suspended at 27 Klett units in chemotaxis buffer. Respiratory rate was measured by oxygen consumption using a Clark electrode. Normally, an experiment was begun by putting the electrode into a bacterial suspension to close it off from the atmosphere and to obtain an initial rate. After about 2.5 min, when oxygen tension fell to about 60 to 70% of saturation, reagent was introduced through a narrow port. The experiment was usually continued until oxygen tension fell to 30 or 40% of saturation. Rates were obtained from slopes of lines expressing oxygen tension as function of time. Measurements were made at 30 C. Control experiments show that changes of about 2% are significant.

ATP measurements. Experiments for adenosine 5'-triphosphate (ATP) determinations were carried out as described (11) using luciferin scintillation.

Abbreviations used are as follows: FCCP, trifluoromethoxycarbonylcyanidephenylhydrazone; CCCP, *m*-chlorocarbonylcyanidephenylhydrazone; CCP, carbonylcyanidephenylhydrazone; *p*COOH-CCP, *p*-carboxycarbonylcyanidephenylhydrazone; PCP, pentachlorophenol; TCSA, 3,3',4',5-tetrachlorosalicylanilide; DBP, 2,6-dibromophenol; DNP, 2,4-dinitrophenol; DCCD, dicyclohexylcarbodiimide; and HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide.

RESULTS

Response to membrane-active reagents. In 1975, Ordal and Goldman (10) reported that certain uncouplers of oxidative phosphorylation and inhibitors of electron transport repelled *B. subtilis* in a spatial gradient assay in which bacteria travel down a gradient of reagent into a capillary tube. They extended these observations to show that the behavioral basis of this migration was increase in tumbling frequency when the reagent increased in concentration (10, 11). In particular they showed that bacteria subjected to reagent tumbled at first, then later resumed normal frequency of swimming and tumbling. However, when the reagent was diluted away, there was at most a fleeting response. These authors indicated that the local anesthetics tetracaine, procaine, and lidocaine caused transient tumbling as well (11). It now appears that chlorpromazine, a central nervous system depressant, also causes temporary tumbling in *B. subtilis* (Fig. 1).

Binding to specific sites. According to the chemiosmotic hypothesis, uncouplers of oxidative phosphorylation cause a decrease in membrane potential by penetrating the membrane as neutral species and returning as anions, each leaving a proton behind (4, 9, 12). For a bacterium or mitochondrion having an electric potential, negative inside, this activity of uncouplers diminishes the potential difference across the membrane. A dispute has arisen about whether uncouplers require specific carriers for their action, and Hanstein and Hafeti (3) have obtained evidence for one or several proteins in mitochondria that are labeled when a radioactive, photosensitive uncoupler is irradiated. In the presence of other uncouplers, the photosensitive uncoupler cannot bind to mitochondria, a result interpreted as indicating displacement from a specific site.

To find out whether uncouplers, inhibitors, and membrane-active drugs mediate their effect through specific sites or, alternatively, by simply diffusing through the membrane, as en-

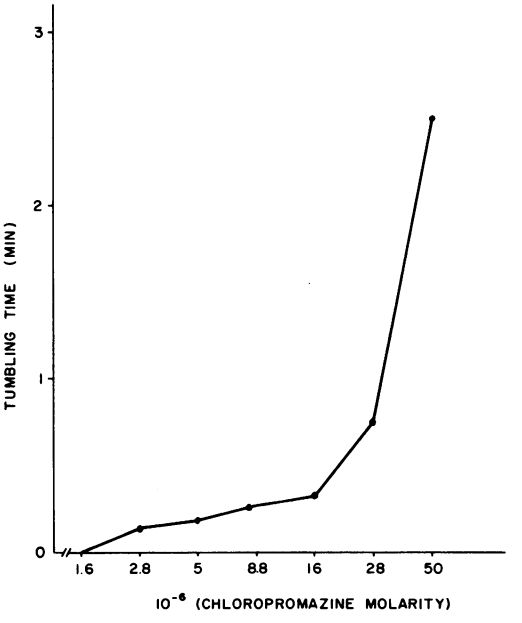

FIG. 1. *Response of bacteria to chlorpromazine. See Materials and Methods for procedure. Chlorpromazine is added at time zero. Concentration of chlorpromazine is graphed on the abscissa; time for bacteria to return to approximately the original frequency of tumbling and swimming is graphed on the ordinate.*

visaged in the chemiosmotic interpretation of uncouplers, the following experiments were done. Bacteria were preincubated in reagent and allowed to return to their natural frequency of swimming and tumbling. Then they were subjected to various concentrations of the same reagent to determine the minimum concentration that produces tumbling, defined as postadaptive threshold to distinguish it from the true threshold, which is the minimum concentration that causes tumbling for untreated cells. This experiment was repeated at several preincubation concentrations of reagent. The rationale of this approach was that if a specific site for interaction with reagent existed, then by preincubating bacteria in that reagent, one would partly titrate this site and make it necessary to add more reagent to these cells than to untreated cells to cause tumbling. Furthermore, if there is just one type of site, then there should be some minimum fraction of sites that need to become bound with reagent to see a response. Assuming that this fraction is the same regardless of the preincubation concentration, one can then calculate the dissociation constant for the interaction of reagent with this site (see Appendix for formula and method of calculation).

These experiments were done with the reagents given in Table 1. Most chemicals showed evidence of saturation—presence of reagent raised the threshold (minimum concentration of reagent causing a response). Table 1 also gives the fraction of binding sites that become complexed when fresh reagent is added: this number is calculated from the dissociation constant given in Table 2.

It can be seen that this fraction is independent of concentration. However, in some instances there is more scatter in the number than in others: the degree of scatter is reflected in the standard deviations to percentage of binding protein given in Table 2. This scatter presumably reflects subjective error in distinguishing the minimum concentration of repellent to cause "decidedly" more tumbling than occurs on addition of buffer (in blind experiments). The fact that 1.8-fold intervals of concentration were used, rather than smaller ones, also contributes to this scatter. In one case, PCP it was apparent that the data fit two dissociation constants much better than one.

CCCP, although an analogue of FCCP (see Discussion), showed by contrast only the slightest hint of saturation: presence of the threshold concentration of CCCP slightly decreased the sensitivity to CCCP, although with higher concentrations, the postadaptive threshold returned to the original value. Results with TCSA were particularly unusual. Apparently, presence of TCSA sensitizes the bacteria to responding to more TCSA. This effect reached a maximum at 1.8×10^{-8} M TCSA, the threshold concentration, and preincubation of bacteria in higher concentrations reduced sensitivity to TCSA (i.e., evidence of saturation). Assuming that the bacteria are fully sensitive to TCSA when preincubated in 1.8×10^{-8} M TCSA, one can calculate a dissociation constant. Results derived from this calculation are given in parentheses in Tables 1 and 2.

Distinctness of sites. To determine specificities of these sites, experiments similar to those reported above were done: bacteria were preincubated in one reagent and tested for shift of postadaptive threshold of a different one. If two chemicals mutually shifted each other's postadaptive thresholds, they probably bind to the same or at least partially overlapping sites. If two drugs have little effect on each other's postadaptive thresholds, then the corresponding sites are distinct. To obtain the clearest results possible, fairly high preincubation concentrations were chosen.

Results of these experiments for chemically dissimilar compounds are given in Table 3. Instances of change of postadaptive threshold by more than 1.8-fold are given in italics. There were no cases of substantial mutual changes of postadaptive threshold, indicating individuality of receptors. However, there were several instances of increased or decreased sensitization of the bacteria to reagent. For instance, tetracaine decreased sensitivity to FCCP, and TPB decreased sensitivity to cyanide but increased sensitivity to chlorpromazine. One explanation of this phenomenon is that one reagent alters the membrane in such a way as to produce more sites or make more sites available for a second reagent.

Similar preincubation experiments were then done to ascertain whether even chemically similar substances cause tumbling through the same sites. The data of Table 4 show that tetracaine and lidocaine raise each other's postadaptive thresholds greatly; therefore, they share approximately the same site. Procaine appears to interact with a partially overlapping site. Figure 2 shows that the structure of procaine is dissimilar from that of lidocaine and tetracaine in having an ionizable unsubstituted amino group para to the ester substituent. Table 5 similarly shows that PCP raises the postadaptive threshold for dinitrophenol and for dibromophenol, but FCCP, a chemically dissimilar uncoupler, does not. Table 6 gives evidence that

TABLE 1. *Effect of preincubation with reagent on postadaptive threshold*

Reagent	Preincubation[a]	Postadaptive threshold[a,b]	Fraction[c]	Reagent	Preincubation[a]	Postadaptive threshold[a,b]	Fraction[c]
FCCP	0	1	0.14	Tetracaine	0	1	0.17
	1	1	0.11		1	1	0.12
	1.8	1.8	0.14		1.8	1.8	0.15
	3.2	3.2	0.17		3.2	3.2	0.17
	5.6	5.6	0.14		5.6	5.6	0.16
	10	10	0.14		10	10	0.13
	18	18	0.11				
				Lidocaine	0	1	0.14
CCCP	0	1			1	1	0.11
	1	1.8			1.8	1.8	0.14
	1.8	1			3.2	1.8	0.11
	3.2	1			5.6	3.2	0.11
	5.6	1			10	10	0.14
CCP	0	1	0.10	Procaine	0	1	0.09
	1	1	0.08		1	1	0.08
	1.8	1	0.07		1.8	1.8	0.11
	3.2	3.2	0.15		3.2	1.8	0.09
	3.2	3.2	0.15		5.6	3.2	0.11
	5.6	3.2	0.11		10	5.6	0.11
	10	3.2	0.07		18	10	0.09
					32	18	0.07
PCP	0	1	0.14				
	1	1	0.11	TCSA	0	1	
	1.8	1.8	0.14		0.1	1	
	3.2	3.2	0.17		0.18	0.56	
	5.6	3.2	0.09		0.32	0.56	
	10	18	0.20		0.56	0.32	
	18	18	0.11		1	0.18	(0.021)[e]
	32	10	0.061[d]		1.8	0.32	(0.030)[e]
	56	10	0.041[d]		3.2	0.32	(0.022)[e]
	100	32	0.065[d]				
				NaCN	0	1	0.032
Chlorpromazine	0	1	0.11		1	1	0.030
	1.8	1	0.08		1.8	1	0.029
	3.2	1.8	0.10		3.2	1	0.026
	5.6	3.2	0.11		5.6	1.8	0.032
					10	1.8	0.032
					18	1.8	0.023
				TPB	0	1	0.17
					1	1	0.12
					1.8	1.8	0.15
					3.2	3.2	0.17
					5.6	5.6	0.16
					10	18	0.18

[a] To convert to concentration, multiply number in column by concentration listed under "Threshold" in Table 2 for the particular reagent.

[b] "Postadaptive threshold" refers to minimum concentration of reagent causing tumbling for bacteria preincubated in a given concentration of the same reagent.

[c] "Fraction" refers to the fraction of hypothetical recognition sites that becomes newly complexed with reagent. It is calculated from the dissociation constant given in Table 2. See Appendix for method of calculation. See text for explanation of parentheses.

[d] Calculated using dissociation constant of 7×10^{-6} M PCP.

[e] Parentheses emphasize tentativeness of calculation. See text.

TABLE 2. *Determination of dissociation constants*

Reagent	Threshold[a]	Dissociation constant[b]	% Binding protein[b,c]	Glutamate (G) or lactate (L)[d]
FCCP	1×10^{-8} M	6×10^{-8} M	13.5 ± 2.1	L
CCCP	1.8×10^{-7} M			L
CCP	1.8×10^{-6} M	1.6×10^{-5} M	9.7 ± 3.1	L
PCP	1×10^{-7} M	6×10^{-7} M	13.6 ± 3.9	L
		7×10^{-6} M[e]	5.6 ± 1.3[e]	
Chlorpromazine	2.8×10^{-6} M	2.2×10^{-5} M	9.7 ± 1.7	G
Tetracaine	9.4×10^{-6} M	4.7×10^{-5} M	15.1 ± 2.1	G
Lidocaine	1.0×10^{-3} M	6.2×10^{-3} M	12.6 ± 1.9	G
Procaine	5.8×10^{-4} M	5.8×10^{-3} M	9.4 ± 1.5	G
TCSA	1.8×10^{-8} M	(1.1×10^{-7}M)[f]	(2.5 ± 0.5)[f]	L
NaCN	5.6×10^{-5} M	1.7×10^{-3} M	2.9 ± 0.4	L
TPB	3.2×10^{-6} M	1.6×10^{-5}M	15.9 ± 1.0	L

[a] In absence of preincubated reagent.
[b] See Appendix for method of calculation.
[c] Standard error of mean.
[d] Indicates whether energy source in buffer was glutamate or lactate.
[e] Second dissociation constant. See text.
[f] Parentheses indicate tentativenss of calculation. See text.

TABLE 3. *Effect of preincubation with one reagent on postadaptive thresholds of others*

Preincubation reagent	Newly added reagent[a]							Concn of preincubation reagent
	NaCN	FCCP	PCP	TCSA	TPB	Chlorpromazine	Tetracaine	
NaCN	1.8	1	1	1	1	1	1	5.6×10^{-4} M
FCCP	0.56	*18*	1.8	1	1.8	1	1.8	1.8×10^{-7} M
PCP	1.8	1	*56*	1	1	1.8	*3.2*	3.2×10^{-6} M
TCSA	0.56	1.8	1	*0.32*	1	1.8	*3.2*	3.2×10^{-8} M
TPB	*3.2*	1.8	1.8	0.56	*18*	*0.32*	1	5.6×10^{-4} M
Chlorpromazine	1.8	1	0.56	0.56	1	*10*	1.8	9.0×10^{-6} M
Tetracaine	0.56	*5.6*	1	1.8	1	1.8	*32*	2.9×10^{-4} M

[a] To convert to concentration, multiply number in column by concentration listed under "Threshold" in Table 2 for the particular reagent. Numbers in italics indicate shifts of postsynaptic threshold by 3.2-fold or more.

TABLE 4. *Effect of preincubation on postadaptive threshold of local anaesthetics*

Preincubation reagent	Newly added reagent[a]			Concn of preincubation reagent
	Tetracaine	Procaine	Lidocaine	
Tetracaine	32	10	>18	2.9×10^{-4} M
Procaine	3.2	18	3.2	1.8×10^{-2} M
Lidocaine	32	5.6	18	1.8×10^{-2} M

[a] To convert to concentration, multiply number in column by concentration listed under "Threshold" in Table 2 for the particular reagent.

FCCP and CCP share nearly the same site but that CCCP does not. In fact, CCP appears to sensitize the bacterium to CCCP. Figure 2 shows, however, that the structures of these compounds are similar, and the reason for the unusual response of the bacteria to CCCP is not understood.

Effect of cyanide. According to Table 2, cyanide interacts with a site whose dissociation constant is 1.7×10^{-3} M. Figure 3, however, shows that cyanide inhibits respiration and that the data fit a curve predicted from interaction with a single component having a dissociation constant of 5×10^{-5} M. Therefore, cyanide does not cause tumbling by virtue of interruption of electron transport.

Although it is not known how cyanide causes tumbling, I offer the following additional observation in the hope that understanding its basis will clarify the chemotactic effect of cyanide. Ordal and Goldman (11) reported that FCCP, an uncoupler of oxidative phosphorylation, causes decrease in ATP levels at high concentrations (10^{-6} M and higher), but that this de-

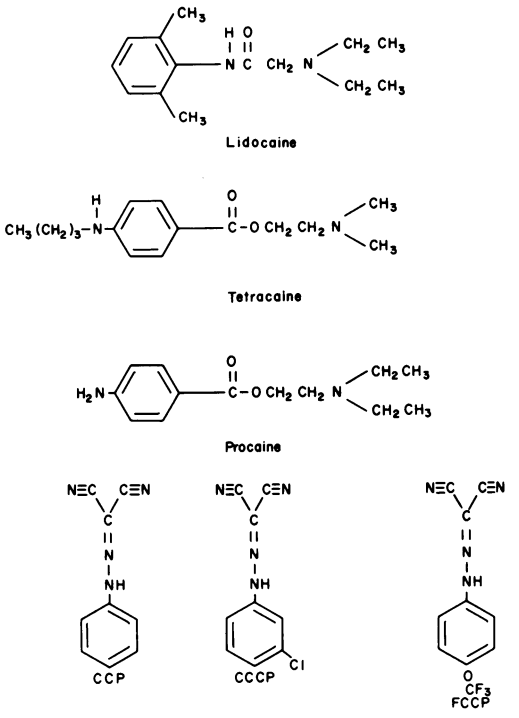

FIG. 2. *Structure of local anaesthetics and CCP derivatives.*

TABLE 5. *Effect of PCP and FCCP on postadaptive thresholds of DNP and DBP*

Preincubation reagent	Newly added reagent		Concn of preincubation reagent
	DNP[a]	DBP[b]	
PCP	3.2	3.2	3.2×10^{-6} M
FCCP	0.56	1	1.8×10^{-7} M

[a] To convert to concentration, multiply number in column by threshold concentration, which is 6.3×10^{-5} M.

[b] To convert to concentration, multiply number in column by threshold concentration, which is 6.3×10^{-6} M.

crease is vastly slowed either by adding DCCD, which prevents the adenosine triphosphatase (ATPase) from coupling ATP levels and the membrane potential (or electron transport), or by using a mutant blocked in such coupling (probably an ATPase mutant). DCCD itself does not much affect ATP levels (Fig. 4); neither does HOQNO, an inhibitor of electron transport (G. W. Ordal, unpublished data). However, cyanide does. In fact, as Fig. 4 shows, use of the mutant or of wild type in presence of DCCD does not prevent cyanide-mediated reduction of ATP levels. Therefore, this reduction occurs by a mechanism independent of the ATPase and possibly independent of electron transport. This mechanism is a mystery.

DISCUSSION

If a finite fraction of binding sites need to become complexed with reagent to give a response (i.e., tumbling for repellents, smooth swimming for attractants), then presence of the reagent, to which the bacteria have already adapted, will shift the (postadaptive) threshold to higher concentrations. That is, more chemical will have to be added to cells preincubated in it than to untreated cells to cause a response. From values of postapaptive thresholds, one can calculate (see Appendix) a corresponding dissociation constant (or, more precisely, a constant analogous to a Michaelis constant, since it is chemotaxis, a process, rather than a binding that is measured). However, if the sites are distinct and the chemicals do not interact, then

TABLE 6. *Effect of preincubation on postadaptive thresholds of CCP derivatives*

Preincubation reagent	Newly added reagent[a]			Concn of preincubation reagent
	FCCP	CCCP	CCP	
FCCP	18	1	5.6	1.8×10^{-7} M
CCCP	1.8	1	1	5.6×10^{-7} M
CCP	5.6	0.32	5.6	3.2×10^{-5} M

[a] To convert to concentration, multiply number in column by concentration listed under "Threshold" in Table 2 for the particular reagent.

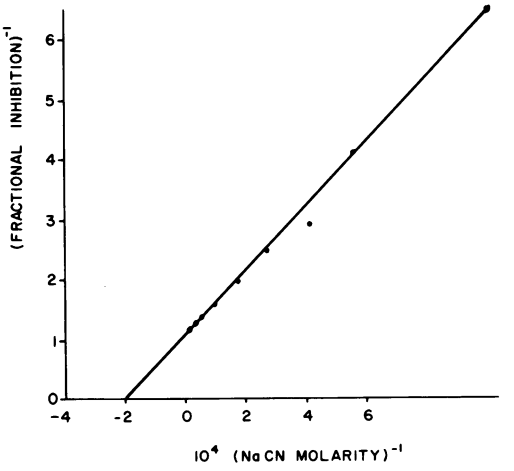

FIG. 3. *Decrease of respiration due to cyanide. See Materials and Methods for procedure. Reciprocal of concentration of cyanide is graphed on the abscissa. Fractional inhibition is one minus ratio of respiratory rate after addition of NaCN to rate before addition of NaCN. Reciprocal of fractional inhibition is graphed on the ordinate.*

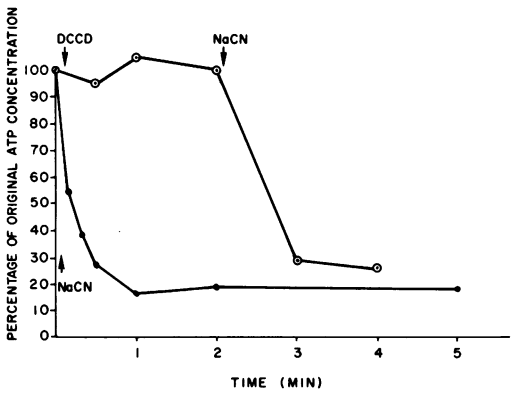

FIG. 4. *Decrease of ATP level due to cyanide. See Materials and Methods for procedure. Time is graphed on the abscissa; percentage of original ATP concentration is graphed on the ordinate. Arrows indicate time of addition of reagent. Upper curve shows experiment with strain OI8. Lower curve shows experiment with OI300, a mutant whose ATP level is not coupled with electron transport (11).*

addition of one chemical to cells preincubated in another should cause a response at the same concentration as for untreated cells. The method is useful in that one can determine whether chemotaxis to a compound is mediated through a receptor; if it is, one can calculate its dissociation constant, determine what other compounds use the same site, and discover how much of the site must become occupied to cause a response.

In general, the picture emerging from these studies is that repellents of *B. subtilis* have recognition sites by which they cause tumbling when added to bacteria. These sites seem different for chemically dissimilar structures, as one might expect. The same site, or at least a partially overlapping site, seems to be used for chemically similar substances, such as the substituted carbonylcyanidephenylhydrazones, on the one hand, or the substituted phenols, on the other. Results with CCCP are anomalous, as explained above. Data from TCSA experiments are unusual in that subthreshold concentrations appear to sensitize the bacteria to further TCSA (see below). PCP may be recognized by two sites. Finally, some reagents somewhat sensitized or desensitized the bacteria to addition of other reagents.

These reagents—local anesthetics and uncouplers of oxidative phosphorylation—are the same ones known in very different contexts, such as nerve cells and mitochondria, but in view of the universality of fundamental patterns in living things, it is plausible that ability to cause *B. subtilis* to tumble is just another manifestation of their respective interactions with membranes. In that context, it is interesting that the local anesthetics tetracaine and lidocaine appear to share a common site, but procaine has only a partially similar one.

The relevance of this work to the mechanism by which uncouplers of oxidative phosphorylation act deserves further comment. Uncouplers are generally considered to act in the same way, since there have never been indications otherwise. As mentioned above, there is a dispute about the requirement for carriers or specific proteins for their activity. The data of this article indicate that the uncouplers TCSA, PCP, and FCCP act at specific sites (analogous to carriers) and that the sites are separate.

Although it is not established that interaction of these reagents with these sites is responsible for altering physiological properties of the bacterium, this appears likely. First, TCSA and FCCP cause increases of respiration in the same range of concentrations that result in tumbling. Second, PCP, although an uncoupler like TCSA and FCCP, does not affect respiratory rate (Ordal, unpublished data), a result that demonstrates that uncouplers do not all act alike. Third, experiments (Ordal and Brummett, unpublished data) indicate that TCSA increases the respiratory rate as its concentration increases much more quickly than does FCCP, implying action by a partially different mechanism. Fourth, FCCP, CCCP, CCP, and *p*-COOH-CCP show parallel effects on tumbling and on uncoupling oxidative phosphorylation in mitochondria—CCP is about 1/100 as effective as FCCP, which starts to affect both at 10^{-8} M, and 1/10 as effective as CCCP, whereas *p*-COOH-CCP is ineffective (5, 10, 11). These results seem too coincidental if the cause of tumbling were altogether unconnected with energetics. Thus, I tentatively conclude that uncouplers act at specific sites in *B. subtilis* and that these sites differ for different types of uncouplers.

APPENDIX

Calculation of dissociation constants from values of postadaptive thresholds is carried out in the following way. Assuming that fraction of site bound with repellent is $S = (R)/(k + R)$, where S is the fraction of sites bound; R, the repellent concentration; and k, the dissociation constant, then the change in fraction of sites bound is,

$$\Delta S = \frac{R_f}{k + R_f} - \frac{R_o}{k + R_o}$$

where R_f is the final repellent concentration and R_o is the original repellent concentration. Manipulation gives,

$$\Delta S = \frac{k(R_f - R_o)}{(k + R_f)(k + R_o)} \quad (1)$$

For each repellent, pairs of R_f, R_o are obtained by determining the minimum concentration of repellent that must be added (ΔR) to bacteria preincubated repellent (R_o) to cause tumbling. $R_f = R_o + \Delta R$.

Then various values of k are assumed. For each, all pairs (R_f, R_o) are substituted into equation 1, and the corresponding values of ΔS are obtained. The value of k that gives the least variation (standard deviation ÷ mean) of these ΔS values is considered the most likely one. This criterion is based on the assumption that the fraction of sites that must become complexed when repellent is added is independent of the fraction bound from preincubation. This assumption has been verified for taxis in *E. coli* and *S. typhimurium* (2, 8, 13).

ACKNOWLEDGMENTS

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