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# Chemotaxis of *Salmonella typhimurium* Toward Citrate

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*Salmonella*, but not *Escherichia coli*, was attracted to citrate, a distinction that is understandable in view of the inability of *E. coli* to transport tricarboxylic acids. The *Salmonella* response to citrate and to two previously described attractants, aspartate and malate, was mutually noncompetitive. Citrate taxis differed from citrate uptake in that it did not require $Na^+$, was constitutive, and was not repressible by glucose.

The enteric bacteria *Salmonella* and *Escherichia coli* closely resemble each other in many ways. However, one fundamental difference that has been noted for many years (5) is the lack, in *E. coli*, of a tricarboxylic acid uptake system or systems, such as *Salmonella* possesses (8, 9). Several years ago, it was noted that citrate attracts *Salmonella* (R. M. Macnab and D. E. Koshland, unpublished data). We have now examined this response in more detail and have found that it appears to be mediated by its own receptor system. We also have found that the response is totally lacking in *E. coli*.

The response to citrate by *Salmonella typhimurium* ST1, an LT2 wild-type strain selected for vigorous motility (4), is shown in Fig. 1. It is evident that cells migrated up gradients of citrate, with a threshold concentration in the capillary assay of ca. $10^{-4}$ M and a maximum response at $10^{-2}$ M.

Citrate is a chelating agent for divalent cations; conceivably, cells could be migrating into capillaries containing citrate because of the reduced concentration of cationic repellents (e.g., $Co^{2+}$ or $Ni^{2+}$ [19]) there, rather than because of an attraction to citrate per se. We have shown in two ways that citrate was attracting the cells directly. First, EDTA was found to be 60-fold less efficient than citrate as an attractant (Table 1), despite its greatly superior chelating power (e.g., the apparent association constants for $Co^{2+}$-citrate and $Co^{2+}$-EDTA are $10^{5.0}$ and $10^{13.5}$, respectively [16]). Second, a uniform background of $10^{-2}$ M EDTA did not reduce the response to a $0 \rightarrow 10^{-2}$ M citrate gradient (Table 2).

The response to citrate was compared with the response to L-aspartate (a potent attractant for *Salmonella* [3, 11]) and several carboxylic acids involved in catabolism (Table 1). Melton et al. (12) recently reported that the peak response of *Salmonella* to L-malate was approximately 4-fold weaker than that to L-aspartate and occurred at a 10-fold-higher concentration. Our results are in agreement with this and establish that malate is intermediate between aspartate and citrate in effectiveness as an attractant. All other compounds tested elicited much lower responses. Figure 2 illustrates the relative potency of aspartate, malate, citrate, and isocitrate over several orders of magnitude in concentration.

Does citrate have its own receptor? Of the known attractants for *Salmonella*, the most obvious candidates to share a receptor with citrate are aspartate and malate. Competition experiments testing these possibilities are presented in Table 2. The data from simple capillary assays showed that if aspartate and citrate shared receptors, $10^{-3}$ M aspartate should drastically reduce a chemotactic response to a $0 \rightarrow 10^{-2}$ M citrate stimulus, and $10^{-2}$ M malate should have a similar effect on the response to a $0 \rightarrow 10^{-3}$ M citrate stimulus. No such competition is evident in the data of Table 2, indicating that citrate possesses a receptor system independent from those for aspartate and malate. In control experiments with $10^{-2}$ M citrate as a competitor to $10^{-3}$ M citrate and $10^{-2}$ M malate as a competitor to $10^{-3}$ M malate, accumulation was reduced to background levels. We found that malate and aspartate did not compete either (Table 2), in agreement with Melton et al. (12) but not with Aksamit et al. (2), who state that a high concentration ($10^{-1}$ M) of malate does inhibit the response to $10^{-3}$ M aspartate. Mesibov and Adler (13) found that an aspartate taxis mutant (AW539) of *E. coli* failed to respond to malate; however, since recent work has revealed that so-called aspartate taxis mutants are actually defective in one of the methyl-accepting chemotaxis proteins (17, 18), this result probably does not reflect competition at the receptor level. Isocitrate, a weak attractant, appears to use the

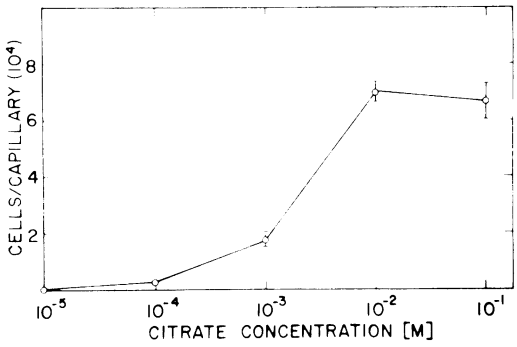


FIG. 1. *Chemotactic response of Salmonella to citrate. Cells of ST1 (wild type) were grown overnight at 37°C in filter-sterilized citrate minimal medium (20) plus 1% (vol/vol) glycerol, suspended in the same medium at $10^7$ cells per ml, grown at 37°C to $5 \times 10^8$ cells per ml, washed twice, and resuspended to a density of $10^7$ cells per ml in motility medium ($10^{-2}$ M phosphate, pH 7, $10^{-4}$ M EDTA [1]), modified by using as counterions $Na^+$-$K^+$ in a 3:1 molar ratio instead of $K^+$ alone. Capillary assays were performed for 30 min at 30°C by the method of Adler (1). Means and standard errors (bars) are based on approximately 20 measurements at each concentration of citrate. The mean background accumulation in the absence of citrate ($6.5 \times 10^2$ cells per capillary) has been subtracted.*

TABLE 1. *Chemotactic response of Salmonella to L-aspartate and carboxylic acids*

| Attractant[a, b] | Chemotactic response[c] (cells per capillary) | |
|---|---|---|
| L-Aspartate | $6.1 \times 10^5$ | $(0.9 \times 10^5)$ |
| L-Malate | $2.2 \times 10^5$ | $(0.3 \times 10^5)$ |
| Citrate | $6.8 \times 10^4$ | $(0.6 \times 10^4)$ |
| Succinate | $2.6 \times 10^3$ | $(1.9 \times 10^3)$ |
| EDTA | $1.2 \times 10^3$ | $(0.3 \times 10^3)$ |
| Fumarate | $7.5 \times 10^2$ | $(1.0 \times 10^2)$ |
| Isocitrate (natural isomer) | $7.4 \times 10^2$ | $(3.0 \times 10^2)$ |
| Oxalate | $3.0 \times 10^2$ | $(2.6 \times 10^2)$ |
| None | $6.5 \times 10^2$ | $(0.5 \times 10^2)$ |

[a] Present in the capillary at $10^{-2}$ M, except for L-aspartate ($10^{-3}$ M).

[b] Sources of chemicals were as follows: L-aspartate, L-malate, isocitrate, succinate, fumarate, and oxalate (Sigma Chemical Co.); citrate (Mallinckrodt); EDTA (Fisher Scientific Co).

[c] Assays were carried out on *Salmonella* ST1, as described in the legend to Fig. 1. Means and standard errors (within parentheses) are based here, and elsewhere, on at least triplicate assays.

citrate receptor (Table 2) since $10^{-2}$ M citrate greatly reduced the response to $10^{-2}$ M isocitrate.

In separate studies investigating responses to divalent cations, expecially $Mg^{2+}$, Ingolia and Koshland (personal communication) have found that *Salmonella* was not attracted to $Mg^{2+}$ per se, but was attracted to magnesium-citrate as a complex, with a higher maximum response and apparent $K_d$ than those for citrate. It is clear that attraction to magnesium-citrate is not the basis of the citrate response, since *Salmonella* responded to citrate in our standard assay medium, which was magnesium-free. Citrate and magnesium-citrate do not appear to be in competition for the same receptor either, since a definite capillary accumulation to $0 \rightarrow 10^{-3}$ M magnesium-citrate gradients occurred in the presence of uniform citrate at $5 \times 10^{-2}$ M (data not shown). Ingolia and Koshland have reached the same conclusion by competition experiments, using a temporal gradient assay.

Thus, citrate chemotaxis appears to be mediated by its own chemoreceptor or chemoreceptors, but its relationship to citrate transport as presently described is tenuous. The evidence pertinent to this last statement follows. (i) Citrate is transported in *Salmonella* by a high-affinity system with a $K_m$ of $2 \times 10^{-5}$ M (9), although genetic and other evidence (7, 8) suggests multiple systems. The concentration dependence of citrate taxis (Fig. 1) would appear to indicate a considerably higher $K_d$ for that process. However, the concentration for peak or even half-maximal response in a capillary assay can yield a substantial overestimate of the $K_d$ of the relevant receptor (1). The chemotaxis results therefore do not eliminate the possible implication of a component of the high-affinity uptake system. (ii) $K^+$ is fourfold less effective than $Na^+$ at stimulating citrate uptake in *Salmonella* (9); in confirmation of this, we observed that growth in minimal medium containing citrate as the sole carbon source was, in the absence of $Na^+$, reduced to 10% of the usual rate. In contrast, the chemotactic responses to $10^{-2}$ M citrate in motility medium constructed with either $1.6 \times 10^{-2}$ M $K^+$ or $1.6 \times 10^{-2}$ M $Na^+$ as the counterion were indistinguishable ($5.3 \times 10^4$ and $3.6 \times 10^4$ cells per capillary, respectively). (Note, however, that the requirement for $Na^+$ for the overall process of citrate transport does not necessarily implicate it in recognition before transport.) (iii) Citrate transport in *Salmonella* is induced by citrate to four times the noninduced level (9); yet we found that cells grown in Vogel-Bonner (citrate-free) (contains $10^{-6}$ M EDTA as a chelating agent) medium plus 1% (vol/vol) glycerol responded strongly to citrate even in the presence of chloramphenicol. Accumulations were slightly lower than in control assays with cells grown in Vogel-Bonner (citrate) medium plus 1% (vol/vol) glycerol ($1.8 \times 10^4$ and $3.7 \times 10^4$ cells per capillary, respectively), possibly as a result of a modification of the gradient by me-

TABLE 2. *Competition assays[a] between various attractants for Salmonella*

| Attractant (M) | Competitor (M) | Response to attractant[b] in absence of competitor (cells per capillary) | Response to competitor[b] (cells per capillary) | Response to attractant[b] in presence of competitor[c] (as % of response in its absence) |
|---|---|---|---|---|
| Citrate ($10^{-2}$) | Aspartate ($10^{-3}$) | $6.8 \times 10^4$ | $6.1 \times 10^5$ | 115 |
| Citrate ($10^{-3}$) | Malate ($10^{-2}$) | $1.8 \times 10^4$ | $2.2 \times 10^5$ | 96 |
| Malate ($10^{-3}$) | Citrate ($10^{-2}$) | $2.1 \times 10^4$ | $6.8 \times 10^4$ | 83 |
| Isocitrate ($10^{-2}$) | Citrate ($10^{-2}$) | $7.4 \times 10^2$ | $6.8 \times 10^4$ | 10 |
| Citrate ($10^{-2}$) | EDTA ($10^{-2}$) | $6.8 \times 10^4$ | $1.2 \times 10^3$ | 147[d] |
| Malate ($10^{-3}$) | Aspartate ($10^{-3}$) | $2.1 \times 10^4$ | $6.1 \times 10^5$ | 80 |

[a] Capillary assays were carried out on *Salmonella* ST1 as described in the legend to Fig. 1.
[b] Present in capillary only at specified concentration.
[c] Present in both capillary and pond at specified concentration.
[d] Designed as a test of chelation competition (see text).

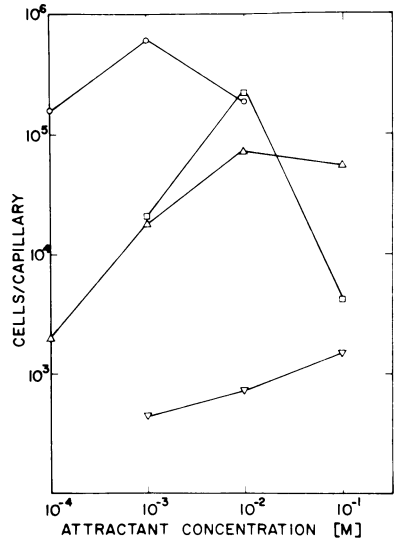


FIG. 2. *Response of Salmonella ST1 to* L*-aspartate* (○), L*-malate* (□), *citrate* (△), *and isocitrate* (▽). *Assays were carried out as described in the legend to Fig. 1.*

tabolism in the latter case. (iv) Glucose strongly inhibits citrate transport. Cells grown in glucose have initial citrate transport rates of some 70-fold lower than cells grown on citrate (9). We found no reduction in taxis to citrate as a consequence of growth on Vogel-Bonner (citrate-free) plus 1% (wt/vol) glucose rather than on Vogel-Bonner (citrate) plus 1% (vol/vol) glycerol ($7.4 \times 10^4$ and $6.8 \times 10^4$ cells per capillary, respectively). From this result and the previous one we conclude that citrate taxis, unlike citrate transport, is constitutive and is not subject to catabolite repression. (v) Two independently isolated fluorocitrate-resistant mutants ($Fc_1^r$ and $Fc_2^r$, obtained from W. W. Kay) gave the same response to citrate as did their fluorocitrate-sensitive parent, Su453 (data not shown). Ingolia and Koshland (personal communication) have also found that three mutants defective in tricarboxylic acid uptake (obtained from K. Imai) have unimpaired citrate taxis. (vi) Repeated attempts by us to isolate citrate taxis mutants by nitrosoguanidine mutagenesis and selection for failure to swarm on semisolid (0.3% agar [Difco Laboratories]) plates containing 10 mM citrate as the sole carbon source were unsuccessful. Transport-negative, taxis-positive mutants should have been screened out by outward migration in the gradient generated by the wild-type population, and transport-positive, taxis-negative or transport-negative, taxis-negative mutants should have remained at the center. Mutants of the latter phenotype would be selected against as growth would have been impossible. The failure to obtain any mutants can therefore be taken as weak evidence linking citrate taxis to citrate transport.

The current situation regarding chemotaxis toward citrate is thus the same as that for chemotaxis toward amino acids and repellents, namely, that although evidence of receptor specificity is available (based on competition experiments), there is no clear correlation with the properties of the corresponding transport system(s), and no receptor mutants have been isolated. Ordal et al. (15) have concluded that taxis and transport can be entirely distinct, as evidenced by inhibition studies of proline recognition in *Bacillus subtilis*.

Citrate taxis has been noted in *Pseudomonas* (14), with a high threshold ($10^{-5}$ to $10^{-4}$ M) and a high concentration ($10^{-2}$ M) for peak response similar to the values that we obtained for *Salmonella*.

*Salmonella* ST1 and two *E. coli* strains, wild type with respect to motility and chemotaxis, were compared with respect to growth rate on, and chemotactic response to, several carbon

TABLE 3. *Ability of Salmonella and E. coli to grow and respond chemotactically to various compounds*

| Compound | Doubling time (h)[a]/accumulation (cells per capillary)[b] | | |
|---|---|---|---|
| | *Salmonella* ST1 | *E. coli* AW405[c] | *E. coli* 20SOK[c] |
| L-Serine | NG/9.7 × $10^4$ | 6.5/3.6 × $10^4$ | NG/7.8 × $10^4$ |
| L-Aspartate | NG/1.6 × $10^5$ | 6.5/2.6 × $10^5$ | NG/1.6 × $10^5$ |
| L-Malate | NG/2.2 × $10^5$ | 1.5/1.4 × $10^3$ | 4.0/0.8 × $10^3$ |
| Citrate | 1.5/6.8 × $10^4$ | NG/0 | NG/0 |

[a] Growth was measured at 37°C in minimal medium (8) plus the carbon source at 10 mM. NG, No measurable increase in turbidity after 24 h from an initial inoculum of ca. $10^7$ cells per ml.

[b] Assayed as described in the legend to Fig. 1. Attractant concentration was $10^{-4}$ M (serine and aspartate) or $10^{-2}$ M (malate and citrate).

[c] *E. coli* strains received from J. Adler.

sources (Table 3). Although growth on L-aspartate or L-serine as the sole carbon source was poor or nonexistent, both compounds acted as excellent attractants for both species. L-Malate was a stronger attractant for *Salmonella* than for either of the two strains of *E. coli*; yet there was a distinct response in the latter species (13). In contrast, citrate, which supports growth in, and acts as an attractant for, *Salmonella* was totally ineffective in either role for the two *E. coli* strains.

What does the lack of a tactic response to citrate in *E. coli* mean? Because of the lack of a relevant uptake system, migration to citrate would be futile; therefore, there should be no selection pressure for the evolution of a citrate chemoreceptor or for the retention of a preexisting one. For this reason alone, it would have been surprising if *E. coli* could respond. This argument, although probably valid, would seem to be replaced by an even stronger one, namely, that it is likely (by analogy with sugar receptors [see, e.g., reviews in references 6 and 10]) that the citrate chemoreceptor in *Salmonella* is also a component of a citrate uptake system and that it evolved in that role first and later evolved further to participate in the system of sensory transduction.

The correlation in *E. coli* between total lack of citrate taxis and total inability to take up citrate reinforces the conclusion reached above, that for *Salmonella*, citrate is a primary attractant, rather than an analog of some more potent, but as yet unidentified, chemical.

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