Participation of the β -Ketoadipate Transport System in Chemotaxis

By MAHMOUD KARIMIAN AND L. NICHOLAS ORNSTON*

Department of Biology, Yale University, New Haven, Connecticut 06520, U.S.A.

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β -Ketoadipate serves as a chemoattractant for *Pseudomonas putida*. The chemotactic response is inducible, and a regulatory mutant strain that forms the β -ketoadipate transport system at high levels exhibits a heightened chemotactic response to β -ketoadipate. Adipate and succinate, compounds that interact with the transport system, inhibit chemotaxis toward β -ketoadipate. Some, but not all, mutants that fail to respond chemotactically to β -ketoadipate lack the β -ketoadipate transport system. It thus appears that the transport of β -ketoadipate is associated with its function as a chemoattractant. It is likely that the metabolite attracts fluorescent *Pseudomonas* species to environments in which complex aromatic polymers undergo microbial dissimulation.

INTRODUCTION

Members of fluorescent *Pseudomonas* species form an inducible transport system that acts on β -ketoadipate (Ornston & Parke, 1976). The system is not formed at a level sufficient to permit the efficient utilization of exogenous β -ketoadipate as a growth substrate (Stanier, 1950; Ondrako & Ornston, 1980). Nevertheless, the system appears to have selective value because it has been conserved in divergent *Pseudomonas* species (Ondrako & Ornston, 1980). In this report we summarize evidence leading to the conclusion that the transport system has been conserved in evolution because it is a component of a mechanism that allows β -ketoadipate to act as a chemoattractant.

METHODS

Strains. Wild-type *Pseudomonas putida* strain PRS2000 and the constitutive mutant strain PRS2178 have been described elsewhere (Ondrako & Ornston, 1980). The regulatory mutation in strain PRS2178 causes elevated expression of the β -ketoadipate transport system and constitutive synthesis of the three enzymes that give rise to β -ketoadipate from β -carboxy-*cis,cis*-muconate (Parke & Ornston, 1976). Mutant strains deficient in β -ketoadipate taxis were selected from strain PRS2178 on the basis of their inability to swarm in response to gradients created on semi-solid agar plates (Armstrong *et al.*, 1967) containing 10 mM- β -ketoadipate. Cells that failed to migrate from the centre of the plates were placed on semi-solid agar plates containing 10 mM-citrate. Cells that formed a ring in response to the citrate gradient were picked, grown in liquid culture with glucose, and spotted once more on semi-solid agar plates containing 10 mM- β -ketoadipate. After repeated transfers, reduced swarming and dense bacterial growth at the origin were observed on the β -ketoadipate plates. Organisms from the origin were purified by single colony isolation on non-selective media; the isolated strains were examined for chemotaxis, the presence of the β -ketoadipate transport system, and constitutive synthesis of the three enzymes that give rise to β -ketoadipate from β -carboxy-*cis,cis*-muconate.

Culture conditions. Cells were maintained at 30 °C during growth and chemotaxis experiments. Cultures grown in defined medium (Ornston & Stanier, 1966) were harvested by centrifugation for 10 min at 3000 g, washed twice with chemotaxis buffer (0.05 M-sodium phosphate buffer containing 10 μ M-EDTA, pH 7.0) and resuspended in chemotaxis buffer. Chemotaxis was observed qualitatively as the formation of expanding rings of growth in response to chemical gradients created on semi-solid agar plates containing defined growth medium and 0.3% (w/v) Difco agar (Armstrong *et al.*, 1967). The plates were maintained in a water-saturated incubator.

Fig. 1. (a) Induction and repression of β -ketoadipate taxis in wild-type *P. putida* strain PRS2000 grown with *p*-hydroxybenzoate ($\circ$), succinate ($\square$) or glucose ($\bullet$). A relative response of 1 corresponds to 5×10^4 cells per capillary, the number observed in capillaries lacking chemoattractant.

(b) β -Ketoadipate taxis of the uninduced transport-constitutive mutant strain PRS2178 grown with glucose ($\circ$), compared with the induced wild-type strain PRS2000 grown with *p*-hydroxybenzoate ($\bullet$).

(c) Inhibition of β -ketoadipate taxis of glucose-grown strain PRS2178 by 0.4 mM-adipate ($\circ$) and 1 mM-succinate ($\bullet$), compared with taxis in the absence of inhibitor ($\square$).

Quantitative chemotaxis assay. Washed cultures were diluted to a concentration of 1.7×10^7 cells ml^{-1} in chemotaxis buffer. Capillary assays were performed for 30 min at 30 °C by the method of Adler (1973). The reported number of cells in capillaries is the average of five determinations. Cells were counted as colony-forming units on succinate plates.

Assay of enzymes and the β -ketoadipate transport system. The specific activities of enzymes were determined by previously described procedures (Ornston, 1966). Radioactive adipate, a non-metabolizable analogue of β -ketoadipate, was used to measure the activity of the β -ketoadipate transport system (Ondrako & Ornston, 1980).

RESULTS

Chemotaxis toward β -ketoadipate is inducible in wild-type bacteria. The β -ketoadipate transport system is induced by growth of wild-type *P. putida* with *p*-hydroxybenzoate, a compound that is metabolized via β -ketoadipate; synthesis of the transport system is repressed by growth with succinate (Parke & Ornston, 1976). As shown in Fig. 1(a), wild-type *P. putida* responded chemotactically to β -ketoadipate, and the tactic response was induced by growth with *p*-hydroxybenzoate. A significant chemotactic response was observed with cells that had been grown with the non-repressing carbon source glucose, whereas succinate-grown cells did not respond chemotactically to β -ketoadipate (Fig. 1a).

Mutations causing heightened β -ketoadipate transport activity lead to an elevated chemotactic response. Glucose-grown cultures of the constitutive mutant strain PRS2178 form the β -ketoadipate transport system at a level about 10-fold higher than that found in induced wild-type cells (Parke & Ornston, 1976). The regulatory mutation in strain PRS2178 also caused a heightened chemotactic response to β -ketoadipate. As shown in Fig. 1(b), the response of uninduced constitutive cells was higher than that of the induced wild-type cells. The threshold of response to β -ketoadipate was somewhat lower than $10 \mu\text{M}$ (Fig. 1b). This concentration does not differ greatly from $40 \mu\text{M}$, the K_i observed with β -ketoadipate as a competitive inhibitor of adipate transport by the system (Ondrako & Ornston, 1980).

Table 1. *Taxis and transport in mutant strains of P. putida*

Mutant strains were selected from strain PRS2178 on the basis of their inability to migrate in response to a gradient of β -keto adipate. All the strains exhibited citrate and succinate taxis on semi-solid agar plates. The taxis mutants, like their parental strain PRS2178, formed constitutively the three enzymes that convert β -carboxy-*cis,cis*-muconate to β -keto adipate. Taxis was determined (5 determinations) with 10 μ M- β -keto adipate, and transport activity was determined with [14 C] adipate.

Strain	β -Keto adipate taxis ($10^{-4} \times$ no. of cells per capillary $\pm$ s.d.)	Rate of adipate transport [pmol (mg dry wt) $^{-1}$ min $^{-1}$]
PRS2178	56 ± 2.2	350
PRS2261	5 ± 0.5	10
PRS2262	6 ± 0.6	18
PRS2263	5 ± 0.5	320

Adipate and succinate inhibit β -keto adipate chemotaxis. Adipate and succinate both interact with the β -keto adipate transport system. Adipate is a non-metabolizable analogue that normally is used to measure the activity of the transport system in *P. putida* (Ornston & Parke, 1976); the K_m of the system for adipate is about 0.2 mM (Ondrako & Ornston, 1980). Succinate competitively inhibits the transport system with a K_i of 1.3 mM (Ondrako & Ornston, 1980). As shown in Fig. 1 (c), both 0.4 mM-adipate and 1 mM-succinate inhibited the chemotactic response of *P. putida* to β -keto adipate.

Selection for absence of the chemotactic response yields mutant strains lacking the transport system. Taxis-deficient mutant strains were selected from strain PRS2178 on the basis of their inability to respond chemotactically to β -keto adipate. Two of the mutant strains had dysfunctions in the β -keto adipate transport system (Table 1). Another mutant strain was defective in β -keto adipate taxis but retained an active β -keto adipate transport system (Table 1). Thus it appears that some activities required for β -keto adipate taxis may be traced directly to the transport system and that one or more additional components are associated with the tactic response.

DISCUSSION

Kinetic, physiological and genetic evidence points to the conclusion that the β -keto adipate transport system is a component of a mechanism that allows β -keto adipate to serve as a chemoattractant for fluorescent *Pseudomonas* species. The activity of the β -keto adipate transport system may be recovered in membrane vesicles (Ondrako & Ornston, 1980). Therefore the common β -keto adipate taxis-transport component cannot be a periplasmic binding protein of the type associated with carbohydrate taxis and transport in coliform bacteria (Hazelbauer & Adler, 1971; Aksamit & Koshland, 1974; Hazelbauer, 1975). By analogy with the membrane-associated phosphotransferase enzyme for glucose (Adler & Epstein, 1974), it might be suggested that the transport of β -keto adipate is vectorial, forming a product like β -keto adipyl-CoA. This seems unlikely because the vesicular transport of β -keto adipate may be driven by respiratory energy in the absence of CoA (Ondrako & Ornston, 1980). Furthermore, mutations that block the intracellular conversion of β -keto adipate to β -keto adipyl-CoA prevent the utilization of exogenous β -keto adipate (Wallis, Ondrako & Ornston, unpublished observations). Thus it appears that the component shared in β -keto adipate transport and taxis is unique among known systems.

Little direct nutritional benefit is conferred by the β -keto adipate transport system, and its primary selective value probably lies in the contribution it makes to chemotaxis. This raises the question of why the bacteria are attracted to a poor growth substrate like β -keto adipate. The answer may lie in the fact that β -keto adipate accumulates as aromatic compounds are dissimilated by microbial cultures (Kilby, 1951). β -Keto adipate may confer

an indirect nutritional benefit by serving as a relatively simple chemical signal attracting fluorescent pseudomonads to environments in which the microbial breakdown of complex aromatic polymers is underway.

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