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Isolation and Characterization of Acetate Kinase and Phosphotransacetylase Mutants of *Escherichia coli* and *Salmonella typhimurium*

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Mutations in the *ack* (acetate kinase) and *pta* (phosphotransacetylase) genes in *Salmonella typhimurium* were characterized and determined to be analogous to those of previously described *Escherichia coli* mutants. We established that in both bacterial species these genes were cotransducible with the neighboring histidine transport operon and were distally located relative to *purF*. *pta* mutants were sensitive to the dye alizarin yellow and were unable to grow on medium containing inositol as a carbon source. We selected mutants of both species with deletions covering both the *ack* and the *pta* genes; some deletions extended into the histidine transport operon.

Two genetic loci, *ack* and *pta*, which encode the enzymes acetate kinase (ATP:acetate phosphotransferase, EC 2.7.2.1 [reaction 1]) and phosphotransacetylase (acetyl-coenzyme A [CoA]: orthophosphate acetyltransferase, EC 2.3.1.8 [reaction 2]), respectively, which together synthesize acetylphosphate, were recently identified in *Escherichia coli* and mapped near *purF*, at 49 min on the linkage map (3-5).

$$\text{acetate} + \text{ATP} \rightleftarrows \text{acetylphosphate} + \text{ADP} \quad (1)$$

$$\text{acetylphosphate} + \text{CoA.SH} \rightleftarrows \text{acetyl-CoA} + P_i \quad (2)$$

Acetylphosphate is thought to be an intermediate in the activation of acetate to acetyl-CoA (4). Also, it has recently been implicated as the energy source for transport systems utilizing periplasmic binding proteins (7).

The region of the chromosome where the *ack* and *pta* genes are located also contains genes which encode histidine transport proteins. In *Salmonella typhimurium*, transport of histidine by the high-affinity system (a binding protein-dependent system) requires products of three genes, *hisJ*, *hisQ*, and *hisP*. These three genes, together with a regulatory locus *dhuA*, form an operon which maps at 48.5 min on the *S. typhimurium* map, near the *purF* locus (2). *E. coli* possesses an analogous histidine transport system with genetic components which are linked to the *purF* locus on the *E. coli* map (Ardeshir and Ames, manuscript in preparation). Thus, this region of the chromosome is of interest to us for two reasons: first, because of the possible involvement of the *ack* and *pta* gene products in energizing histidine transport, and second, because the *ack*/*pta* genes provide useful genetic markers closely linked to the histidine transport operon. We have isolated and further characterized *pta* and *ack* mutants of both *S. typhimurium* and *E. coli*. We describe new properties of bacteria defective in these genes and we have mapped the region more carefully.

Independent mutants resistant to 15 mM monofluoroacetate (FAc) (4) were selected on minimal salts medium (6) plates containing 10^{-2} M NH_4Cl as the nitrogen source and either 0.3% pyruvate or 0.3% lactate as the sole carbon source. (Inhibition of *S. typhimurium* by the FAc was also obtained on plates containing citrate [0.4%] as a carbon source. A single resistant mutant isolated under these conditions was analyzed and did not map near *hisP*. It was not studied further.) For both *S. typhimurium* and *E. coli* two classes of fluoroacetate-resistant (FAc^r) mutants were obtained. One class consisted of *pta* mutants which were defective in phosphotransacetylase (PTA) activity; we discovered that this class was sensitive to the monoazo dye alizarin yellow GG (AY; Matheson, Coleman, and Bell). The other class, consisting of *ack* mutants was only slightly sensitive to AY as compared with the wild-type strain and was defective in acetate kinase (ACK) activity. Table 1 shows the correlation between the enzyme assays and the phenotypic properties of representative mutants. There was a complete correlation between loss of PTA activity and sensitivity to AY. Of 21 FAc^r strains (TA3492 to TA3513) of *S. typhimurium*, 9 (TA3492 to TA3509 and TA3511 to TA3500) were sensitive to AY and phenotypically identical to TA3492 (PTA^- ACK^+), and 10 (TA3501 to TA3509 and TA3511) were partially sensitive to AY and behaved like TA3501 (PTA^+ ACK^-); the remaining

TABLE 1. *Properties of ack and pta mutants in S. typhimurium and E. coli*[a]

Strain[b]	Sensitivity		Enzyme level		Gene assignment
	FAc[c]	AY[d]	PTA[e]	ACK[f]	
S. typhimurium					
TA831	S	R	1.2	1.4	Wild type
TA3492	R	S	<0.05	1.3	*pta*
TA3501	R	PS	1.2	0.2	*ack*
TA3159	R	S	<0.05	0.2	*his* Δ(*pta-ack-dhuA-hisJ-hisQ*)*6628*
TA3512[g]	R	S	−	−	*pta* Δ(*pta-ack-dhuA-hisJ-hisQ-hisP*)*10*
E. coli					
TA3512	S	R	+	+	Wild type
TA3514	R	S	−	+	*pta*
TA3515	R	PS	+	−	*ack*
TA3516[g]	R	S	−	−	*pta* Δ(*pta-ack-hisQ-hisP*)
TA3476[g]	R	S	−	−	*his* Δ(*pta-ack-dhuA-hisJ-hisQ-hisP*)

[a] All enzymatic assays were carried out at 37°C. Total protein of cell extracts was determined by the method of Lowry (10), with bovine serum albumin as standard.

[b] The *S. typhimurium* parent was either TA831 (Δ*hisF645*) or its derivative TA271 (Δ*hisF645 dhuA1*), and the *E. coli* parent was either TA3522 [Δ(*hisOGDCBHAFIE-gnd*)*461 dhuA1*] or TA3521 [Δ(*hisOGDCBHAFIE-gnd*)*461 dhuA2*].

[c] S, FAc sensitivity (wild-type phenotype); R, FAc resistance (mutant phenotype), determined by streaking cells on minimal salts plates (6) containing 0.3% sodium pyruvate as the carbon source and 15 mM FAc (Calbiochem) and scoring growth after 24 to 36 h of incubation at 37°C.

[d] AY sensitivity was determined by inspection of colony size after 24 to 36 h of incubation at 37°C on tryptone plates containing 0.25% AY. The wild type (R) can be clearly distinguished from a partially sensitive *ack* mutant (PS) and from sensitive *pta* and *pta ack* mutants (S). These distinctions are more evident in *S. typhimurium* than in *E. coli.* On an AY plate, sensitive colonies are darker, as well as smaller, than AY-resistant colonies, in rough proportion to the degree of sensitivity, thus indicating perhaps a difference in extent of uptake of the dye.

[e] Quantitative PTA assays were performed by the method of Brown et al. (4). Specific activities are reported in micromoles of NADH formed per minute per milligram of protein. Qualitative PTA activity was determined by the disappearance of acetylphosphate in the presence of CoA as follows. The reaction mixtures contained 14 mM lithium acetylphosphate (Sigma Chemical Co.), 0.57 mM CoA, 5.7 mM mercaptoethanol, 29 mM oxaloacetic acid, 140 mM Tris-hydrochloride (pH 7.4), and 0.014 U of citrate synthase (Sigma) per ml. Acetylphosphate was determined by the method of Lipmann and Tuttle (9). The results of these qualitative assays were visually estimated as "+" for the wild-type activity, or "−" if no color change was evident.

[f] Quantitative ACK assays were carried out by the method of Janson et al. (8), and specific activities are reported in micromoles of NAD^+ formed per minute per milligram of protein. All *ack* strains assayed had specific activities of 0.2 U of ACK per mg of protein, which is probably due to some other ATPase activity present in the cell extracts. For strains whose ACK activities are given as "+" or "−," the method of Rose et al. (12) was used.

[g] Selected as simultaneously resistant to HIPA and FAc (see text).

2 strains (TA3512 and TA3513) lacked both PTA and ACK and were sensitive to AY.

For *E. coli,* eight FAc^r mutants were isolated (TA3514 to TA3520 and TA3476). One of these (TA3514) was sensitive to AY and lacked PTA activity; one was PTA^+ ACK^- (TA3515) and partially sensitive to AY; and six PTA^- ACK^- strains (TA3516 to TA3520 and TA3476) were sensitive to AY. Therefore, in both *S. typhimurium* and *E. coli,* AY sensitivity persists in double *pta ack* mutants.

Table 1 also shows the properties of some *S. typhimurium* and *E. coli* deletion mutants which lack both enzymes. Nine such mutants (of which TA3159 is a representative) already existed in our collection of *S. typhimurium* transport-deficient strains (Fig. 1). They carry deletions extending from the transport operon into both the *ack* and *pta* genes. No deletion mutation is presently available which extends into only one of these genes. The existence of these deletion mutants demonstrates that the lack of both PTA and ACK activities is not lethal and that no essential genes are encoded in the region between the transport operon and the *ack/pta* genes. These strains have no nutritional requirement as they are able to grow on minimal medium, although slightly less well than the wild type.

The response to AY allows a quick screening procedure for classifying FAc^r strains as ACK deficient or PTA deficient. Several other dyes structurally related to AY were screened in an effort to understand the mechanism of inhibition and to obtain a more powerful inhibitory dye. The following dyes were tested at an approxi-

mate final concentration of 0.03% in tryptone-yeast extract agar and found not to inhibit *ack* and *pta* mutants significantly: trypan red, Biebrich scarlet, Evans blue, amaranth, lissamine fast red, congo red, Ponceau S, *p*-(*p*-aminophenylazo)benzenesulfonic acid, 8-anilino-1-naphthalenesulfonic acid, orange II, Ponceau 3R, and Bordeaux red. Crystal scarlet was found to slightly inhibit *ack* mutants but not the wild-type or *pta* mutants. The physiological significance of AY inhibition has yet to be clarified.

The *pta* and *ack* mutations were mapped by phage P1-mediated transduction in *E. coli* and P22-mediated transduction in *S. typhimurium*. In each of these species both genes were linked to the *purF* locus at 49 min (Table 2) on the chromosomal map (3, 14). This location agrees with previously reported data for *E. coli* (4, 5). In *S. typhimurium*, the histidine transport operon has been shown to be 40% linked to *purF* by P22-mediated transduction (2). Similarly, histidine transport mutations in *E. coli* were shown to be linked to *purF* (98% by P1 transduction; Table 2). In *S. typhimurium*, FAc^r mutations were found to be 60% linked to Δ(*hisQP*)*6712*; because they are 5% linked to *purF*, the histidine transport operon must lie between the *ack/pta* genes and *purF*. The data obtained with *E. coli* mutants agree with this interpretation (Table 2B).

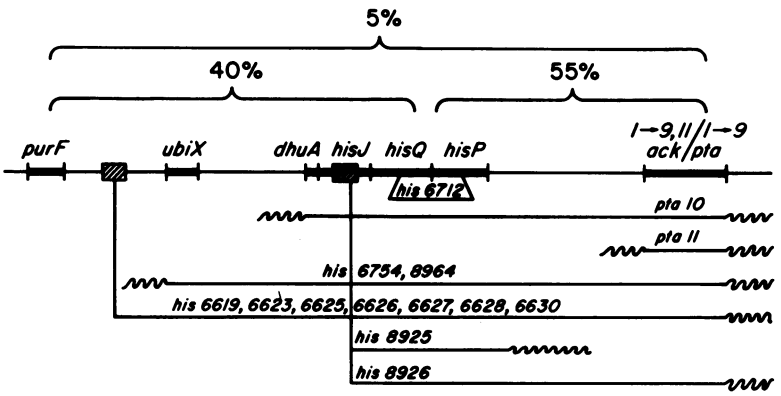

FIG. 1. *Linkage map of S. typhimurium region at 48 units. Map distances are expressed as phage P22-mediated cotransduction frequencies. The relative order of the ack and pta genes has not been determined. The straight lines represent deletions, and the wiggly lines indicate lack of information as to the exact termini of the deletions. The hatched boxes on the chromosome (between purF and ubiX, and in hisJ) are sites of transposon Tn10 insertion (2), and deletions with one endpoint at those sites were originated by Tn10-stimulated deletion formation (11). The numbers are allele numbers. Distances are not drawn to scale. Deletion pta-10 was determined not to extend into the ubiX gene by showing that the growth of a strain carrying this deletion mutation (TA3512) was not stimulated by p-hydroxybenzoic acid (2).*

TABLE 2. *Genetic linkages of pta and ack to purF and the histidine transport operon*

Recipient[a]	Donor	Selected marker[b]	Unselected marker[b] scored	% Linkage
A. Transductions by phage P22 in *S. typhimurium*				
purF145	*ack*	*purF*$^+$	FAc^r	6 (6/100)
purF145	*pta*	*purF*$^+$	FAc^r	5 (5/100)
Δ(*hisQP*)*6712*	*ack*[c]	*hisQP*$^+$	FAc^r	60 (286/479)
Δ(*hisQP*)*6712*	*pta*[c]	*hisQP*$^+$	FAc^r	59 (294/498)
B. Transductions by phage P1 in *E. coli*				
purF	*ack*[d]	*purF*$^+$	FAc^r	62 (29/47)
purF	*pta*[e]	*purF*$^+$	FAc^r	70 (30/43)
purF dhuA^{+f}	*dhuA1*[f]	*purF*$^+$	*dhuA1*[f]	98 (42/43)

[a] Transduction techniques were those described by Roth (13). All strains are histidine auxotrophs.

[b] Only relevant markers are listed.

[c] All *Salmonella* FAc resistance mutations determined are linked to the histidine transport operon; the linkages given are averages of several crosses.

[d] In TA3515.

[e] In TA3514.

[f] Histidine auxotrophs of both *E. coli* and *S. typhimurium* do not grow on D-histidine unless the *dhuA1* mutation in the histidine transport operon, causing elevated D-histidine uptake, is introduced.

Because of the proximity of the *ack/pta* genes to the histidine transport operon, we were able to enrich for deletion mutants by simultaneously selecting for defective histidine transport and resistance to FAc. Strains with mutations in the histidine transport operon are resistant to α-hydrazinoimidazole propionic acid (HIPA), an inhibitory histidine analog transported by the high-affinity histidine transport system (15). Clones simultaneously resistant to HIPA (1 mg on a paper disk) and to 15 mM FAc incorporated in the plate were selected from TA3521, an *E. coli* histidine auxotroph. Lactate (0.4%) was used as the carbon source, and 3×10^{-5} M carnosine was the histidine source. These mutants were tested for the presence of an intact high-affinity histidine transport system by screening for their ability to utilize D-histidine as a source of L-histidine (2). Of 17 independent cultures, 7 yielded clones which had lost the ability to transport D-histidine. Six of these seven (TA3516 to TA3520 and TA3476) were studied further and found to lack both PTA and ACK activities and to be sensitive to AY, suggesting that they contain deletions covering both the *pta* and the *ack* genes and extending into the histidine transport operon. The mutants were screened by sodium dodecyl sulfate-polyacrylamide gel electrophoresis for production of the histidine-binding protein J (1). Two of them (TA3476 and TA3519) lacked the binding protein; all others produced parental levels of the protein. The two clones defective in J protein production (TA3476 and TA3519) were used as recipients in a cross with one of the putative deletion mutants producing J, with selection for recovery of transport function (i.e., growth on D-histidine), to prove that they carried deletions extending from *ack/pta* to at least the *hisJ* gene. If the two clones (TA3476 and TA3519) carried double mutations, in *hisJ* and in *ack/pta*, they should have been able to recombine with the wild-type *hisJ* gene of the donor clone. In fact, neither of the crosses yielded any wild-type transport recombinants, confirming that the two recipient strains carry deletions of part or all of the histidine transport operon.

Several attempts were made to select for simultaneous resistance to HIPA and FAc in *S. typhimurium*. Only 1 culture of 14 tested yielded a clone (TA3512) which was defective in histidine transport besides being FAc^r. This mutant was unable to recombine with several strains carrying mutations in the histidine transport genes and the *dhuA* locus; the TA3512 mutation was therefore classified as a deletion of the *dhuA* and *hisJQP* genes. It is unclear why we obtained FAc^r HIPA^r mutants more readily in *E. coli* than in *S. typhimurium*.

FAc^r mutants were screened for the ability to utilize a variety of carbon sources. It was found that *S. typhimurium ack* mutants grew poorly and that *pta* mutants were unable to grow on inositol (doubling times, 4.2 h and >12 h, respectively, as compared with 1.4 h for the wild type). This observation was first made by B. Bochner. The *E. coli* wild type is unable to grow on inositol. This property allows the selection of wild-type recombinants or revertants from *pta* mutants. Using genetic crosses on inositol, we were able to establish that mutation *pta-11* (in TA3513) was a deletion covering three *pta* mutations which could recombine with each other. This strain was also shown to lack ACK and PTA, but it had an intact histidine transport operon (Fig. 1). Both *pta* and *ack* mutant strains of *S. typhimurium* grew less well (doubling times, 4.2 and 3.5 h) than did the wild type (doubling time, 2.0 h) on medium containing 0.6% acetate as a carbon source. This is in contrast to results obtained with *E. coli pta* mutant strains, which do not grow on acetate (4). These results indicate that *S. typhimurium* must have an alternative route of synthesis of acetyl-CoA from acetate.

For both species, we also obtained FAc^r strains with mutations which mapped near the *purF* locus but had apparently normal levels of both PTA and ACK. Because it is possible that such strains produced either PTA or ACK altered in such a way that they would have lost affinity for the fluoro derivative but not for their normal substrates, we will not classify those strains as a new class of FAc^r analogous to *facB* strains (5) until further studies have proven that these enzymes are indeed unaltered.

In conclusion, we isolated *ack* and *pta* mutants of *E. coli*; we have confirmed the biochemical properties of these genes and accurately established their map location with respect to the histidine transport operon. In addition, we have isolated and characterized analogous mutants of *S. typhimurium* and shown that they have several new properties.

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ADDENDUM IN PROOF

All six PTA$^-$ ACK$^-$ strains (TA3516 to TA3520 and TA3476) were shown by Pascal et al. to be defective for an additional neighboring gene, *nirE*, which is located at 49.5 min on the *E. coli* map and is cotransducible with *purF* (A. Abou-Jaoudé, M. Lepelletier, J. Ratouchniak, M. Chippaux, and M. C. Pascal, Mol.

Gen. Genet. **167**:113–118, 1978). This result confirms that these strains harbor deletion mutations.

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