JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 488-490

Vol. 116, No. 1
Printed in U.S.A.

Osmotically Induced Excretion of Putrescine by Mutants of *Escherichia coli* Defective in Potassium Transport[1]

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Received for publication 7 May 1973

Escherichia coli is known to excrete putrescine rapidly after a sudden elevation in the osmolarity of the medium. We report here that mutants defective in the transport of potassium ions display a greatly diminished rate of [^{14}C]putrescine excretion.

We demonstrated previously that the putrescine content of *Escherichia coli* varies inversely with the osmolarity of the medium (5). A sudden increase in medium osmolarity produces a rapid excretion of putrescine which can be blocked by low K^+ concentrations in the medium (K_m, 0.69 mM K^+). Putrescine excretion (5) and K^+ uptake (3) may both play a role in the adaptation of *E. coli* to media of high osmolarity. We now report that several mutations which reduce K^+ uptake also lead to a reduction in putrescine excretion after an osmotic stress.

Mutations in each of four closely linked cistrons (A, B, C, and D), collectively named the *kdp* system, produce the same phenotype (1, 2); the *kdp* system is expressed only at K^+ concentrations less than 0.1 mM, the growth rate of such strains being half-maximal at 0.05 mM K^+ (W. Epstein, personal communication). Two additional loci, *trkA* and *trkD*, also affect K^+ transport. A strain containing mutations at the *kdp* and *trkA* loci grows at a half-maximal rate in 2.4 mM K^+; a strain containing *kdp*, *trkA*, and *trkD* mutations grows at a half-maximal rate in 17 mM K^+ (W. Epstein, personal communication).

We first measured the ability of mutants in the *kdp* genes (FRAG-5), *kdp* and *trkA* genes (2K133), or *kdp*, *trkA*, and *trkD* genes (2K401m) to excrete [^{14}C]putrescine after a sudden increase in external osmolarity (Fig. 1). Media of high osmolarity, containing 30 mM K^+, elicited rapid [^{14}C]putrescine excretion by both the parent strain FRAG-1 (derived from *E. coli* K-12) and by FRAG-5 (*kdpABC*). Strain 2K133 (*kdpABC*, *trkA*) excreted putrescine more slowly in high osmolarity medium than did either FRAG-1 or FRAG-5. Putrescine excretion by strain 2K401m (*kdpABC*, *trkA*, *trkD*) was slower yet. The apparently rapid loss of putrescine from strain 2K401m in low-osmolarity medium was not a reproducible observation.

The initial rates of [^{14}C]putrescine excretion were next compared in media containing various concentrations of K^+, from 1 to 60 mM (Table 1). When the K^+ concentration was 11 mM or greater, FRAG-1 and FRAG-5 lost putrescine at high rates in response to high osmolarity. At 1 mM K^+, the rates of excretion were about half-maximal for both strains, in agreement with previous results for *E. coli* B (5). However, strains 2K133 and 2K401m excreted [^{14}C]putrescine more slowly than the parent strain (Table 1); these rates of excretion are in reasonable agreement with the known rates of K^+ uptake by these cells (W. Epstein, personal communication). Strain 2K401m achieves a moderate rate of K^+ uptake only at high external K^+ concentrations; similarly, [^{14}C]putrescine is lost very slowly at low and intermediate values of external K^+. Strain 2K133 has the same K^+ uptake process found in strain 2K401m and also contains the *trkD* system (K_m about 0.5 mM). Thus, strains such as 2K133 should and do exhibit moderate rates of putrescine loss at intermediate K^+ concentrations. For both strains, rapid putrescine loss begins at higher K^+ values than would be predicted from the rate of K^+ uptake. Perhaps,

[1] This paper is publication no. 497 of the Department of Biophysics and Genetics, Univ. of Colorado Medical Center, Denver.

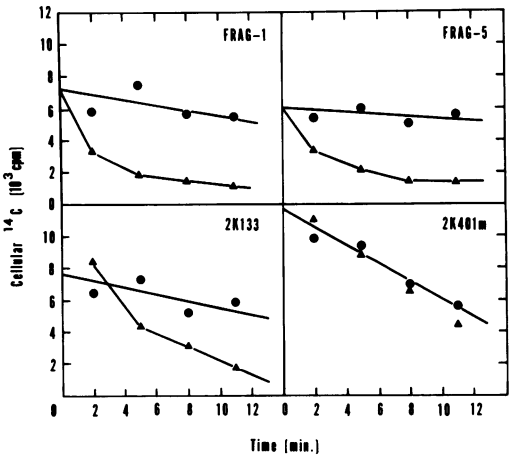

FIG. 1. *Excretion of [^{14}C]putrescine by strains FRAG-1, FRAG-5, 2K133, and 2K401m of E. coli in media of high or low osmolarity. Cultures of each strain were grown to an optical density of 0.400 at 600 nm in 60 mM K^+ medium (5) containing 1 mg of thiamine-hydrochloride per liter (Mann Research Labs.). This K^+ concentration allows all strains to grow at the maximal rate. Samples (5 ml) were incubated for 15 min at 37 C with 1 μCi of [1,4-^{14}C]putrescine·2 hydrochloride (109 μCi/mg, New England Nuclear Corp.), centrifuged at 37 C at about 2,000 × g for 10 min, and washed twice in 5 ml of 30 mM K^+ medium. Cell pellets were then resuspended by vigorous vortexing in 1.5 ml of 30 mM K^+ medium. At 15-s intervals 0.5 ml of culture were added to 9.5 ml of 30 mM K^+ medium (●) or 30 mM K^+-0.632 M sucrose medium (▲; final sucrose concentration, 0.6 M). At various times after addition of cells to media, 1.0-ml samples were removed and pipetted into 1.0 ml of the same medium on ice. Within 10 min the chilled cell suspensions were collected on Schleicher and Schuell filters (type B-6, pore size 0.45 μm, 25 mm in diameter). Filters were then washed with 12 ml of ice cold 0.15 M NaCl and counted in 5 ml of scintillation fluid (5). Samples which were pipetted directly onto filters and washed with warm 0.15 M NaCl produced the same results.*

a rapid rate of K^+ entry is necessary to initiate loss of putrescine.

The putrescine contents of each of the four strains were determined. All strains grown in 60 mM K^+ medium contained high levels of putrescine, similar to those of *E. coli* B (93–118 μmol of total putrescine/g of protein). Similarly, all strains grown in the same medium with the addition of sucrose to 0.6 M contained low levels of putrescine (1.1–13 μmol/g of protein). The spermidine contents were either not affected or slightly reduced by growth in media of high osmolarity.

It is possible that the translocation of putrescine is physically linked to one specific K^+ transport system. If putrescine loss were linked to either the *kdp*, *trkA*, or *trkD* systems (assuming all three are distinct from each other), then mutations in one of these alleles might have drastic effects on the rate of putrescine loss. However, it appears that each mutation has a progressive effect on the rate of putrescine excretion. If putrescine loss were linked to some other K^+ transport system not studied here, then mutations in the *trkA* and *trkD* systems should have no effect on putrescine loss. Since they do affect the rate of excretion, the translocation mechanism for putrescine is probably linked to the rate of entry of K^+ into the cell and not to one specific K^+ transport system.

TABLE 1. *Effect of external potassium concentration on the osmotically induced loss of [^{14}C]putrescine by mutants of E. coli*[a]

Strain	Genotype	% of intracellular ^{14}C lost in 5 min in response to high osmolarity (0.6 M sucrose)			
		1 mM[b]	11 mM[b]	30 mM[b]	60 mM[b]
FRAG-1	Wild type	38	75	71	68
FRAG-5	*kdpABC5*	33	73	71	62
2K133	*kdpABC5 trkA133*	0	8.6	34	40
2K401m	*kdpABC5 trkA401 trkD1*	—	0	3.1	30

[a] Bacteria for all experiments were grown to an optical density of approximately 0.400 in 60 mM K^+ medium containing 1 mg of thiamine-hydrochloride per liter. Cultures were labeled with [^{14}C]putrescine as described in the legend to Fig. 1, washed with low-osmolarity media of the K^+ concentration shown in the table, and resuspended in the same media either with or without 0.6 M sucrose present. Percent losses were estimated at 5 min after osmotic stress as the difference between ^{14}C in high- and low-osmolarity cultures.

[b] K^+ concentration.

This conclusion is strengthened by the observations of Rubenstein et al. (6). These authors described enhanced synthesis and excretion of putrescine by K^+-starved mutants of *E. coli* which are leaky for K^+. One of these mutants is known to take up K^+ from the medium at an increased rate (4). As shown here, mutants deficient in transport of K^+ lose putrescine at a reduced rate. Thus, studies with both types of mutants are consistent with the interpretation that the rate of putrescine loss is dependent on the rate of K^+ uptake.

We are indebted to Wolfgang Epstein of the Department of Biochemistry, University of Chicago, for the gift of strains FRAG-1, FRAG-5, 2K133, and 2K401m, and for discussion

with us of his experiments with these strains. We thank Frank Harold for valuable discussions during the course of this work and Kitty Hercules and Judith L. Munro for critical reading of the manuscript.

This research was supported by National Science Foundation grants GB-21413 and GB-8267.

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