BBA 71001

Abnormal phosphorus metabolism in a potassium transport mutant of *Escherichia coli*

The recent discovery of microbial mutants with defects in K^+ accumulation provides a unique opportunity to explore the mechanism of ion accumulation with a fresh perspective. Such mutants have been reported by LUBIN AND KESSEL[2], DAMADIAN AND SOLOMON[1], SLAYMAN AND TATUM[3], LUBOCHINSKY, MEURY AND STOLKOWSKI[4] and DAMADIAN[5].

Because of the common laboratory experience that ion transport is keenly dependent on cellular metabolism, there are numerous reports in the literature that link ion accumulation to phosphorus metabolism. None of the potassium mutants, however, has been shown to possess abnormalities of phosphorus metabolism that could be related to their impairment in K^+ transport. It is the purpose of this paper to report that our K^+ transport mutant of *Escherichia coli* (strain RD-2*) does not take up P_i normally.

In each experiment, single colony isolates of parent strain (CBH) and mutant (RD-2) were grown overnight in tryptic digest medium (Balt. Biol. Labs.), harvested by centrifugation, and washed with 300 mM sucrose to remove medium phosphate. Cells were then resuspended in a K^+ and P_i "free" medium (pH 7.4) containing 100 mM Tris–HCl and 8 mM $(NH_4)_2SO_4$. The turbidity of the suspension was adjusted to 60 Klett spectrophotometer units. The K^+ present in the medium from the impurities in water and reagents was measured with an Instrumentation Laboratories flame photometer. It varied between 10 and 20 μmoles/l of medium. Any phosphorus present was less than could be detected with the phosphorus method of CHEN, TORIBARA AND WARNER[6] (4.5 μM). Since there is a small but uniformly present population of revertants in each mutant culture, the revertant density of the cell suspension (number of revertants per mutant cell) was determined for each experiment. The revertant density never exceeded $1 \cdot 10^{-6}$.

$^{32}P_i$ uptake was studied by measuring cell radioactivity at various intervals after the initiation of uptake by an injection of $Na_2H^{32}PO_4$–glucose into the washed cell suspension. The cells in 1-ml aliquots were collected on 25-mm Millipore filters (0.45 μ), washed with two 5-ml portions of a 300 mM sucrose–1 mM Na_2HPO_4 wash solution and counted.

The results demonstrate that in a medium low in both K^+ and P_i, the mutant accumulates $^{32}P_i$ at a rate that varies from $^1/_2$ to $^1/_3$ of the parent's uptake rate as medium P_i is varied (Fig. 1a). This impairment of $^{32}P_i$ accumulation in the mutant is associated with its K^+-accumulating defect[5]. The reduced rate of $^{32}P_i$ uptake in low-K^+–low-P_i medium is accompanied by a reduction in the rate of K^+ uptake. At the start of the experiments cell K^+ was 2.6 $\pm$ 0.4 mM (S.E.) for CBH and 1.1 $\pm$ 0.40 mM for RD-2. 15 min after injection of $Na_2H^{32}PO_4$–glucose cell K^+ values were 63.45 $\pm$ 0.04 mM and 24.625 $\pm$ 0.005 mM for CBH and RD-2, respectively. Cell K^+ was determined by methods described in a previous publication[5]. The total phosphorus

* Strain RD-2 is now one of four potassium transport mutants that we have isolated. Three of these were produced with ultraviolet light as the mutagenic agent and one (RD-2) was produced with nitroguanidine.

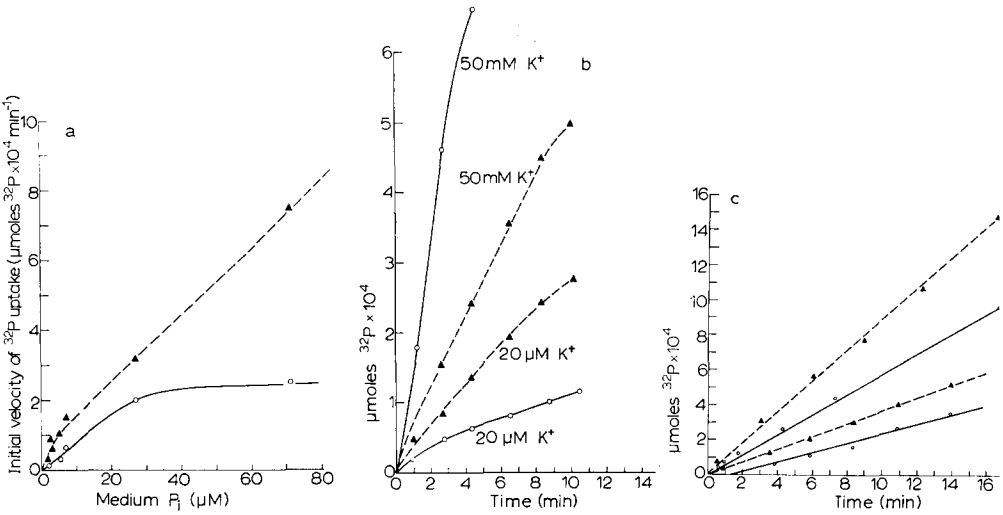

Fig. 1. $^{32}P_i$ uptake from low-phosphate medium by K^+ transport mutant (strain RD-2) and control strain (CBH). Resting cells of the two strains in a final volume of 10 ml were incubated with shaking for 20 min at 37° in Tris–HCl–$(NH_4)_2SO_4$ medium (see text). 1 ml of $Na_2H^{32}PO_4$ (New England Nuclear Corp.; 30 mC/ml) in 10% glucose was then added and 1-ml samples were taken at the indicated times for analysis. Each sample contained 110 μg of cells (dry weight). Sample activities were greater than ten times background. They were counted in an end-window gas-flow counter with an efficiency of 53.3% for $^{32}P_i$. The minimum total count was 2540. Medium P_i concentration values are t_0 values computed from the amount of P_i that is added in the glucose–$^{32}P_i$ injections. The P_i of the glucose–$Na_2H^{32}PO_4$ solution was measured by the method of CHEN, TORIBARA AND WARNER[6]. The medium lacks histidine, a nutritional requirement of both strains. ▲- - -▲, parent strain (CBH); ○—○, mutant (RD-2). (a): The initial velocity of $^{32}P_i$ uptake in low-K^+–low-P_i medium as a function of medium $[P_i]$. Medium P_i concentration varied by varying the amount of $Na_2H^{32}PO_4$ added. K^+ concn. is 20 μM. The $^{32}P_i$ uptake rates of the two strains did not differ when medium P_i was greater than 0.5 mM. (b) and (c): $^{32}P_i$ uptake in low-K^+–low-P_i medium; the effect of added K^+ and Na^+. Medium P_i concn. is 1.8 μM in (b) and 40 μM in (c). 20 μM K^+ designates low-K^+–low-P_i medium without added K^+ or Na^+.

content of the two strains at the start of the $^{32}P_i$ uptake differed by only 4%. The values were 959 ± 9.6 for CBH and 919 ± 8.47 for RD-2 (μmoles/g dry wt.). Total phosphorus was determined by the method of BARTLETT[7] on cells that had been washed twice in normal saline to remove organic compounds present in the medium.

When KCl was added to the low-K^+–low-P_i suspension to raise the K^+ concentration to 50 mM, it produced the unexpected result shown in Fig. 1b. $^{32}P_i$ uptake of the mutant increased 10-fold, whereas the parent strain only increased 1.75-fold, resulting in a mutant phosphorus uptake that was three times more rapid than parent strain uptake. In a similar experiment with the addition of 50 mM NaCl instead of KCl, the low phosphate uptake was inhibited (Fig. 1c). The stimulating effect of K^+ is therefore not the effect of change in medium ionic strength produced by the added salt. K^+ stimulation of $^{32}P_i$ uptake in *E. coli* has been described by ROBERTS AND ROBERTS[8] and this result is consistent with their findings. In our experiments the increased rate of $^{32}P_i$ accumulation produced by K^+ was associated with an increased rate of cell K^+ uptake. In contrast to the results given above for K^+ accumulation during $^{32}P_i$ uptake in low-K^+–low-P_i medium, $^{32}P_i$ uptake in KCl supplemented medium was accompanied by a more rapid increase in cell K^+. In the same

time interval, cell K^+ increased from the same initial levels to 120 ± 1 mM in CBH and 110 ± 1 mM in RD-2.

The K^+ stimulation and Na^+ inhibition manifested by this $^{32}P_i$ uptake reaction are reminiscent of the effects that these ions have on the K^+-stimulated systems that have been studied *in vitro* (KACHMAR AND BOYER[9] and MUDD AND CANTONI[10]). The great stimulation of $^{32}P_i$ uptake that K^+ produces in the mutant is unusual, but at this stage is still unexplained.

In sum, we have found that our K^+-accumulating mutant (strain RD-2) does not take up P_i normally. The mutant's $^{32}P_i$ uptake reaction is greatly stimulated by K^+ and is inhibited by Na^+, characteristics which resemble the behavior of the K^+-stimulated enzymes that are most commonly found catalyzing reactions in intermediate metabolism that involve the transfer of a phosphate group. Perhaps the mutant's K^+ transport abnormality will eventually be traced to a K^+-requiring enzyme that is essential to phosphorus metabolism.

The author is indebted to his research assistant Mr. CARL PAYNE for skillfully performing many of the $^{32}P_i$ uptake experiments, to Lt. Col. HAROLD L. BITTER whose interest and encouragement have contributed significantly to these studies, and to Dr. JAMES WILLIS who suggested the total phosphorus measurements that initiated these studies in phosphorus metabolism.

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Received February 20th, 1967

Biochim. Biophys. Acta, 135 (1967) 378–380