Inhibition of Potassium Transport by Sodium in a Mutant of *Streptococcus faecalis**

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ABSTRACT: A mutant of *Streptococcus faecalis* was isolated which required elevated levels of K^+ for growth at pH 7.5, but only in media rich in Na^+. Growth was inhibited by 300 mM Na^+ at pH 7.5 but not at pH 6. The process sensitive to inhibition by Na^+ appears to be K^+ transport. At alkaline pH both net uptake of K^+ and ^{86}Rb–Rb^+ exchange were much more strongly inhibited by Na^+ in the mutant than in the wild type. However, both strains retain K^+ equally well when incubated with 200 mM Na^+ at pH 7.5.

The mutation has no significant effect upon the uptake of K^+ and Rb^+ when present alone, but affects interaction of the transport system with Na^+. The findings are in accord with our previous suggestion that sites involved in the selective entry of cations into the cells are distinct from those required for their retention.

Like other microorganisms, *Streptococcus faecalis* accumulates K^+ and Rb^+ during growth, even in media containing Na^+ as the predominant cation. The selective transport of cations is under genetic control and mutants have been isolated which are defective in this process. We have recently described mutants of *S. faecalis* which required high concentrations of K^+ or Rb^+ for growth at pH 6 but were indistinguishable from the wild type at pH 8. The defect was found to reside in the retention of K^+ and Rb^+. At pH 6, the mutant cells lost these cations to the medium by exchange for external Na^+. The selective transport of K^+ and Rb^+ inward was essentially normal (Harold *et al.*, 1967).

The present paper describes a complementary phenotype. Mutants of class Tr_{K8}^- require high concentrations of K^+ or Rb^+ for growth at pH 8, but only in the presence of excess Na^+. It will be shown that in these mutants the entry of K^+ and Rb^+ into the cells is abnormally sensitive to inhibition by Na^+.

Materials and Methods

Organisms and Growth Media. The media used to grow *S. faecalis* strain 9790 have been described earlier (Harold *et al.*, 1967; Harold and Baarda, 1967). (*i*) *Defined media.* NaM is buffered with sodium maleate (350 mM Na^+) and supplemented with K^+ or Rb^+ as needed. Similarly, medium KM is buffered with potassium maleate (200 mM K^+) and medium Tris-M with Tris-maleate; these media contain no Na^+. Whenever necessary the pH was maintained by addition of NaOH, KOH, or Tris. (*ii*) *Complex media.* The tryptone–yeast extract media described by Abrams (1960) and Zarlengo and Schultz (1966) are designated K-TY and Na-TY, respectively.

Isolation of Mutants. Mutants were isolated by an adaptation of the ^{32}P-suicide method described previously (Harold *et al.*, 1965). Cells were irradiated and grown overnight in medium KM. The washed cells were then grown for 6 hr in NaM medium (pH 7.5) containing 0.2 mM K^+ and 0.16 mM [^{32}P]Pi (sp act. 3100 $\mu c/\mu mole$). At the end of this period the cells were collected, resuspended in nonradioactive medium containing 20% glycerol, and stored at −70°. After 1 month the great majority of the cells had been inactivated. The survivors were plated on KM plates, replicated onto NaM plates (1 mM K^+, pH 7.5), and clones that failed to grow on the latter medium were picked. All the experiments described here were performed with descendants of a single clone (576B). This mutant has a high reversion rate and had to be recloned repeatedly.

Other Procedures. To study ^{86}Rb–Rb^+ exchange, cells were grown on medium NaM–Rb, resuspended in Tris-maleate buffer, and incubated with $^{86}RbCl$ of known specific activity. Samples were collected by Millipore filtration and washed with 2 mM $MgCl_2$. Sodium, potassium, and rubidium were determined by flame photometry. In some experiments the cells were allowed to glycolyze in water, and a constant pH was maintained with a Radiometer pH-Stat. All the procedures were described in detail in an earlier paper (Harold *et al.*, 1967).

Results

Growth of Mutant 576B. Mutant 576B differed from the wild type in being unable to grow on medium NaM (2 mM K^+ or Rb^+) at pH 7.5. Since this medium

* From the Division of Research, National Jewish Hospital, and Department of Microbiology, University of Colorado School of Medicine, Denver, Colorado. *Received May 22, 1967.* This investigation was supported in part by U. S. Public Health Service Research Grant AI-03568.

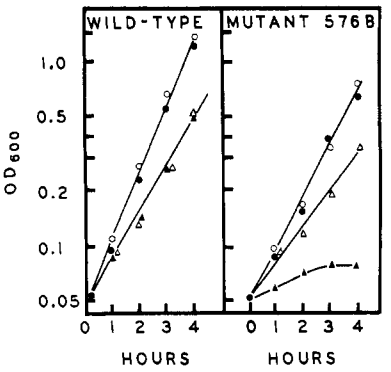

FIGURE 1: Effect of NaCl on growth of wild type and mutant 576B. Both strains were grown overnight on KM medium. The cells were washed and introduced into medium Tris-M containing 1 mM K^+. (○) pH 6, no additions; (●) pH 6, 300 mM NaCl; (△) pH 7.5, no additions; (▲) pH 7.5, 300 mM NaCl.

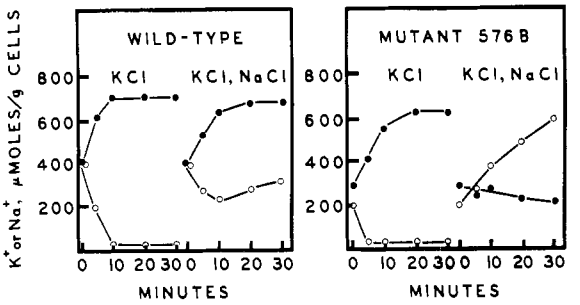

FIGURE 2: Inhibition of net K^+ uptake by Na^+. Wild type and mutant 576B were grown on medium Na-TY overnight. The cells were washed with water, resuspended in water, and allowed to glycolyze at 37° with 10 mg/ml of glucose. The pH was maintained at 8 by means of a pH-Stat. At 0 min KCl (2 mM) or KCl plus NaCl (200 mM) were added as shown. (●) K^+; (○) Na^+.

contains 350 mM Na^+ which is known to be a competitive inhibitor of K^+ and Rb^+ uptake (MacLeod and Snell, 1948; Abrams, 1960; Harold *et al.*, 1967), we compared the effects of Na^+ on the growth of mutant and wild type in Tris medium. As shown in Figure 1, the mutant grew on 1 mM K^+ regardless of the pH. Addition of 300 mM NaCl had little effect at pH 6 but strongly inhibited growth at pH 7.5. The inhibition of growth could be overcome by raising the concentration of K^+. Growth of the wild type was unaffected by this concentration of NaCl at either pH. It thus appears that the defect in 576B is one that renders this mutant sensitive to inhibition by Na^+.

Inhibition of Net K^+ Uptake by Na^+. Cells of *S. faecalis* grown overnight on medium Na-TY are relatively depleted of K^+ and contain large amounts of Na^+ and H^+. In the presence of an energy source such cells take up K^+ with concurrent extrusion of Na^+ and H^+ (Zarlengo and Schultz, 1966; Harold *et al.*, 1967). The effects of NaCl on these processes in wild type and mutant 576B are compared in Figure 2. The two strains behaved identically in the absence of Na^+. In the wild type, addition of 200 mM NaCl at pH 8 had little effect on K^+ uptake but extrusion of Na^+ from the cells was incomplete under these conditions. Uptake of K^+ by the mutant was completely blocked and the cells accumulated Na^+ instead. The rates of glycolysis were equal in all.

Inhibition of ^{86}Rb–Rb^+ Exchange by Na^+. Cells of *S. faecalis* harvested from medium NaM–Rb contain Rb^+ as the major cation. In the presence of an energy source such cells carry out uptake of ^{86}Rb by autologous exchange for internal Rb^+ (Harold *et al.*, 1967). In both wild type and mutant 576B the exchange was inhibited by Na^+, but inhibition was more pronounced in the mutant. For example, in an experiment in which both strains were incubated with 1 mM ^{86}Rb in Tris buffer at pH 8, the concentration of Na^+ required to reduce the initial rate of ^{86}Rb uptake by one-half was 75 mM in the wild type but only 25 mM in the mutant. At pH 6, about 150 mM Na^+ was required for both strains.

Figure 3 is a Lineweaver–Burk plot showing the effect of Na^+ upon the initial rate of ^{86}Rb uptake in the two strains. The data for the wild type can be summarized by the statement that Na^+ acts essentially as a competitive inhibitor of ^{86}Rb uptake, with but little effect upon V_{max} (see also Abrams, 1960). In absence of Na^+ the apparent K_m was 0.17 mM; 50 mM Na^+ increased this to 0.67 mM, from which the K_i for Na^+ was calculated to be about 17 mM. The situation in the mutant is more complex, in that both K_m and V_{max} were affected by Na^+. In the absence of Na^+ the kinetic parameters of rubidium uptake by 576B were the same as those in the wild type (K_m 0.17 mM; V_{max} 15 μmoles/g of dry cells per min). Na^+ at 50 mM increased the apparent K_m to 1.3 mM but also reduced V_{max} to 7 μmoles/g of dry cells per min.

Efflux of K^+ and Rb^+. Wild type and mutant were grown on medium K-TY. Potassium-loaded cells were filtered, washed, and incubated in sodium maleate buffer (200 mM Na^+, pH 7.6) with and without glucose. As shown in Figure 4, there was no significant difference between mutant and wild type with respect to their ability to retain K^+. In both strains K^+ was largely retained for the duration of the experiment, regardless of the presence of an energy source. Retention of K^+ thus does not depend upon continuous reabsorption of K^+ from the external environment.

A difference between wild type and mutant did exist with respect to retention of Rb^+. When cells loaded with ^{86}Rb were incubated in sodium maleate buffer at pH 7.6 (37° glucose present), loss of ^{86}Rb from the mutant was significantly more rapid (50% in 30 min) than from the wild type (50% loss in 60–80 min). It may be significant that, even in the wild type, K^+ and Rb^+ are not equivalent at pH 8. Uptake of K^+

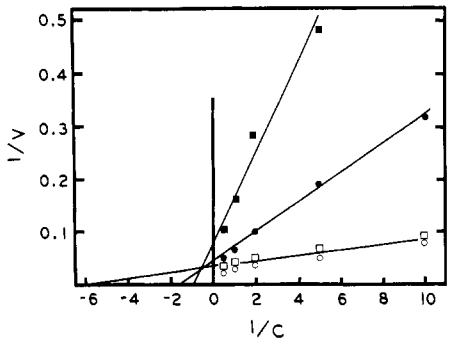

FIGURE 3: Inhibition of ^{86}Rb–Rb^+ exchange by Na^+. Wild type and mutant 576B were grown on medium NaM–Rb. Washed cells were preincubated with glucose at 37° in Tris-maleate buffer (0.1 N, pH 8). After 10 min aliquots were pipetted into prewarmed tubes containing $^{86}RbCl$ and NaCl to give the final concentrations shown. Samples were filtered after 2.0 min. In the reciprocal plot, C is the ^{86}Rb concentration in micromoles per milliliter, V the uptake of ^{86}Rb in μmoles/g of dry cells per 2 min. (○) Wild type, no Na^+; (●) wild type, 50 mM Na^+; (□) mutant, no Na^+; [■] mutant, 50 mM Na^+.

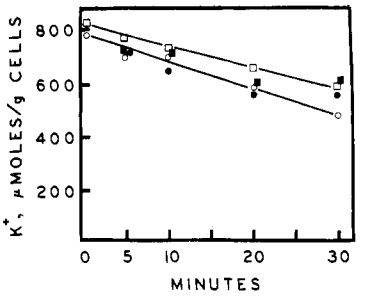

FIGURE 4: Retention of K^+. Wild type and mutant were harvested from medium K-TY. The cells were washed and incubated in 0.1 M sodium maleate buffer (pH 7.6) at 37° (cell density, 0.9 mg/ml). (○) Wild type, no glucose; (●) wild type, plus glucose; (□) mutant, no glucose; (■) mutant, plus glucose.

is more rapid, and retention more complete, than that of Rb^+; moreover, uptake of ^{86}Rb is more strongly inhibited by Na^+ than is K^+ uptake (Figures 2 and 3).

Discussion

Mutant 576B described here was isolated by virtue of its inability to grow on medium NaM at pH 7.5. It was then found that the mutant grew almost normally on Tris medium (1 mM K^+) regardless of pH, but growth at alkaline pH was inhibited by addition of NaCl; the inhibition could be relieved by raising the K^+ content of the medium. Mutant 576B is thus subject to inhibition by Na^+ at concentrations that do not affect growth of the wild type.

From the characteristics of cation exchanges in mutant and wild type it seems likely that the process sensitive to Na^+ inhibition is the transport of K^+ and Rb^+. Mutant and wild type retain K^+ equally well (this is not quite true for retention of Rb^+). However, at pH 8 both net uptake of K^+ by heterologous exchange for internal Na^+ and autologous exchange of ^{86}Rb for internal Rb^+ (Harold *et al.*, 1967) are inhibited more severely in the mutant than in the wild type. In the wild type, Na^+ acts essentially as a competitive inhibitor of ^{86}Rb uptake, whereas in the mutant Na^+ increases the apparent K_m and concurrently reduces the maximal rate of ^{86}Rb uptake (Figure 3). In view of the complexity of the system elaborate deductions from the kinetic data do not seem warranted. We shall content ourselves with the qualitative interpretation that K^+, Rb^+, and Na^+ interact with the transport system at a site which determines selective *entry* of cations into the cells. The mutation affects primarily the interaction with Na^+ but has no significant effect upon the transport of Rb^+ and K^+ in the absence of Na^+.

We have previously proposed that cation exchanges in *S. faecalis* are mediated by a transport system which has at least two specific sites; one site controls cation selection for entry, the other for exit (Harold *et al.*, 1967). In general agreement with this model, the potassium-transport mutants of *S. faecalis* fall into two classes. Mutants designated Cn_{K6}^- select K^+ and Rb^+ over Na^+ for entry as well as does the wild type, but their exit site has high affinity for K^+ and Rb^+; the mutants are therefore unable to retain K^+ and Rb^+ under certain conditions (Harold *et al.*, 1967). Conversely, mutants of class Tr_{K8}^- described here retain K^+ well and thus presumably have a normal exit site, but their entry mechanism is abnormally sensitive to inhibition by Na^+. Two classes of K^+-transport mutants are found in other microorganisms as well. The mutant of *Escherichia coli* originally isolated by Lubin and Ennis (1963) was shown by Günther and Dorn (1966) to have a defective exit mechanism. On the other hand, K^+-requiring mutants of *E. coli* (Damadian, 1966; Damadian and Trout, 1966) and of *Neurospora* (Slayman and Tatum, 1965) were found to have an increased K_m for K^+ transport inward, and presumably have a defective entry site. (Incidentally, the possibility that the elevated K_m for K^+ in the latter mutants may reflect inhibition by Na^+ was not discussed.)

Our findings raise at least two questions which remain unanswered. It will be noted that the two mutant classes found are defective in entry at pH 8 (Tr_{K8}^-) and in retention at pH 6 (Cn_{K6}^-), respectively. The significance of these complementary pH dependencies is unknown. Attempts to isolate additional K^+-transport phenotypes have not been successful. We thus have no information concerning genetic control of cation selection for entry at acid pH or for exit at alkaline pH. Second, we would point out that no K^+-transport mutants have as yet been reported which have *totally* lost the capacity for energy-dependent transport of K^+. Only relatively minor modifications

in selectivity are known to occur. This contrasts with the numerous mutants which appear to have largely or totally lost transport mechanisms for sugars (Egan and Morse, 1966a,b; Hagihara *et al.*, 1963; Winkler and Wilson, 1966; Rickenberg *et al.*, 1956), amino acids (Ames, 1964; Kessel and Lubin, 1962, 1963), or inorganic anions (Dreyfuss, 1964; Pardee *et al.*, 1966; Harold *et al.*, 1965; Harold and Baarda, 1966). Failure to isolate mutants carrying more drastic defects in K^+ transport may indicate that the process is complex and is controlled by multiple genetic loci. More plausibly, total loss of this transport system may be lethal for reasons as yet unknown.

Acknowledgment

It is a pleasure to thank A. Abrams for helpful criticism and for the loan of his pH-Stat.

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