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Energetic consequences of two mutations in *Escherichia coli* K^+ uptake systems for growth under potassium-limited conditions in the chemostat

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The energetics of growth of two *Escherichia coli* strains (TK 2240 and TK 2242) differing in K_m of the high-affinity potassium uptake system and lacking the low-affinity system were studied in the chemostat under potassium-limited conditions. The results were compared with the results obtained previously (Mulder, M.M., Teixeira de Mattos, M.J., Postma, P.W. and Van Dam, K. (1986) Biochim. Biophys. Acta 851, 223–228) with the wild-type FRAG-1, having two potassium uptake systems, and FRAG-5, a mutant which lacks the high-affinity potassium uptake system. We postulated that the high-affinity potassium uptake system was able to generate such a steep gradient across the membrane that the low-affinity system would act in reverse, thus creating a futile cycle of potassium ions at the cost of energy. As a result, FRAG-1 would show a higher ATP turnover at all growth rates tested than the mutant FRAG-5, in which strain the proposed futile cycle is interrupted because of the lack of the high-affinity system. It is shown here that the results obtained with TK 2240 and TK 2242 are in line with our hypothesis of futile potassium cycling. Under our experimental conditions, the yield on potassium was not dependent on the kinetic parameters of the uptake systems. The (thermodynamic) energy demand of the uptake systems determined the carbon substrate conversion required to achieve this yield.

Introduction

Escherichia coli possesses at least two potassium uptake systems, which differ in their affinity for potassium [1]. One is the low-affinity potassium uptake system (Trk) that is constitutively present. Kdp is a high-affinity system that is induced whenever the extracellular potassium concentration drops below values of 1–5 mM [1]. The kinetic parameters of the potassium uptake systems in the various strains used in the study reported in this paper and in the previous paper are summarised in Table I. We postulated that the high-affinity potassium uptake system (Kdp) requires more energy per potassium ion transported than the low-affinity system. The gradient of potassium ions across the membrane thus created by the high-affinity uptake system was much higher than could be generated by the low-affinity system (Trk) alone. We suggested that, as a result, the low-affinity system may start to operate in

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Abbreviations: J_a, anabolic flux; J_{ATP}, ATP turnover; Kdp and Trk, high- and low-affinity potassium uptake system.

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TABLE I

PROPERTIES OF MUTANTS IN POTASSIUM UPTAKE SYSTEMS OF *ESCHERICHIA COLI* K-12

All data concerning FRAG-1 and FRAG-5 as well as the kinetic data concerning TK 2240 and TK 2205 are from Ref. 1. Data on genetics of TK 2240 and TK 2205 are from Ref. 1. All data concerning TK 2242 are from Dr. E.P. Bakker, personal communication.

E. coli: strain	Relevant genotype	Kdp			Trk		
		pheno-type	K_m (mM)	V_{max} ($mmol \cdot g^{-1} \cdot min^{-1}$)	pheno-type	K_m (mM)	V_{max} ($mmol \cdot g^{-1} \cdot min^{-1}$)
FRAG 1		+	0.002	0.15	+	1.5	0.55
FRAG 5	*kdpABC5*	−			+	1.5	0.55
TK 2240	*trkA*405						
	*trkD*1	+	0.002	0.15	±	linearly dependent on extracellular potassium concentration	
TK 2242	*trkA*405						
	*trkD*1						
	kdp-42	+	0.3–0.5	0.15	±	K^+_{out} (mM)	V ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
TK 2205	*trkA*405						
	*trkD*1					20	3
	kdpABC5	−			±	80	12

reverse, thus creating futile cycling of potassium ions as a consequence of the presence of these two systems simultaneously [2]. We showed that *E. coli* FRAG-1, having both uptake systems under potassium-limited conditions, had a higher ATP turnover than FRAG-5, a mutant lacking the high-affinity potassium uptake system, at all growth rates tested [2].

In order to obtain more evidence for the proposed futile cycle of potassium ions [2], a mutant that lacks the low-affinity uptake system was needed to show whether or not the absence of the low-affinity uptake system also interrupts the futile cycling of potassium. Unfortunately, however, no mutants of *E. coli* are known that completely lack the low-affinity system. In this paper we have investigated the growth behaviour of two mutant strains of *E. coli* (TK 2240 and TK 2242) that have mutations in the low-affinity system resulting in a very low activity of this system at the potassium concentration used in our study (Table I). The K_m of the high-affinity system differs 100-fold (0.002 mM for TK 2240 and approx. 0.3–0.5 mM for TK 2242 in liquid media) (Refs. 3 and 4; see also personal communication of Dr. E.P. Bakker). The kinetic parameters of the high-affinity system of TK 2242 are of the same order as the kinetic parameters of the wild-type Trk system. This strain was tested to investigate whether a modified Kdp system would result in the same growth behaviour as that in FRAG-5. We show that the results of growth studies in the chemostat were in agreement with the model of potassium cycling introduced previously [2]. We report that as long as cells were able to take up potassium at a rate commensurate with growth, the yield on potassium was not dependent on the extracellular potassium concentration. It is also shown that a change in the K_m of the high-affinity potassium uptake system did not influence the ATP turnover.

Materials and Methods

Organisms. The following organisms were used in these experiments: *E. coli* TK 2240 (*F⁻ trkD*1 *trkA*405 *nagA lacZ rha thi*) and *E. coli* TK 2205 (*F⁻ trkD*1 *trkA*405 *nagA lacZ rha thi kdpABC*5) [3] (kindly donated by Dr. W. Epstein, University of Chicago) and *E. coli* TK 2242 (*F⁻ trkD*1 *trkA*405 *kdp*-42 *nagA lacZ rha thi*) [5] (kindly donated by Dr. E.P. Bakker, University of Osnabrück).

Growth conditions. All growth conditions and media were the same as described before [2].

Determination of products. This was performed as described in Ref. 2. Calculation of the rate of

ATP production was performed with the same formula as described before [2].

Determination of potassium. 1 ml cell culture was mounted on top of 0.5 ml silicone oil (3/1 mixture of AR200/AR20 Wacker Chemie, München, F.R.G.) in an Eppendorf tube and centrifuged for 30 s in order to separate cells and external fluid. The supernatant was used for the determination of the external potassium. The pellet was resuspended in 500 μl H_2O and afterwards 100 μl perchloric acid (7.5%) was added to determine the internal potassium content. Potassium was measured with a Hitachi atomic absorption spectrophotometer, model 180–80.

Results

E. coli TK 2240 and *E. coli* TK 2242 were grown aerobically in a chemostat under potassium-limited (1 mM) conditions in minimal salts media with glucose as carbon and energy source. The strains have mutations in potassium-uptake systems as summarised in Table I.

As shown in Fig. 1, the bacterial dry weights of TK 2240 and TK 2242 were strongly dependent on the growth rate. There were no gross differences between the two strains. Also, the results were similar to those found previously with *E. coli* FRAG-1 and FRAG-5 [2].

Glucose was used as sole energy and carbon source in these experiments. The glucose is converted to carbon dioxide, acetic acid and biomass. From these products and from our knowledge of the biochemical pathways, the specific ATP production rates could be calculated [2]. In Fig. 2 the specific ATP production rates are shown for two strains. As can be seen, the ATP production rate for TK 2242 is the same as for TK 2240. The lines are described by (best fit by linear regression):

for TK 2240: $J_{ATP} = 224(\pm 23)\cdot(-J_a) + 36(\pm 5)$ mmol/g·h

for TK 2242: $J_{ATP} = 222(\pm 15)\cdot(-J_a) + 33(\pm 4)$ mmol/g·h

The lines relating ATP turnover with growth rate for FRAG-1 and FRAG-5, respectively, were described by [2]:

$J_{ATP} = 332(\pm 11)\cdot(-J_a) + 22(\pm 2)$ mmol/g·h

and

$J_{ATP} = 165(\pm 21)\cdot(-J_a) + 32(\pm 5)$ mmol/g·h

Thus, the results obtained with *E. coli* TK 2240 and TK 2242 are intermediate in between those for FRAG-1 and for FRAG-5.

We also measured the extracellular potassium concentration of the two strains, TK 2240 and TK 2242, as a function of the growth rate. This con-

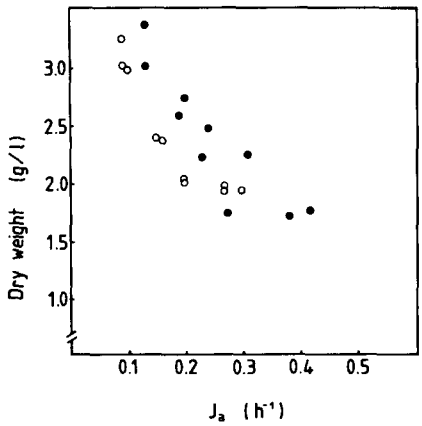

Fig. 1. Relationship between the bacterial dry weight and the growth rate of TK 2240 (○) and TK 2242 (●). J_a = numerical equivalent to dilution rate (D) and is the thermodynamic growth rate.

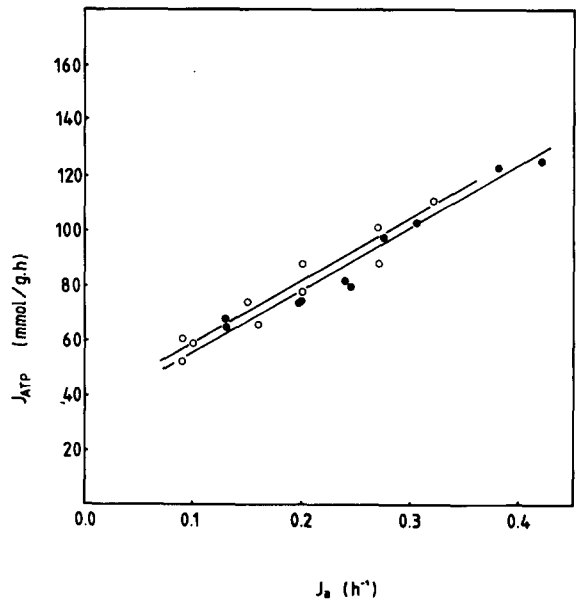

Fig. 2. Relationship between the ATP production rate and the growth rate of TK 2240 (○) and TK 2242 (●).

centration was found to be very low for both strains at all growth rates (less than 0.5 μM). The results obtained were of the same order as those for FRAG-1 [2]. The extracellular potassium concentration for FRAG-5 was higher (25 μM) [2].

We also tested another mutant of *E. coli* (TK 2205) in the chemostat under the same conditions. This strain lacks the high-affinity potassium uptake system. It has the same low activity of the low-affinity system as TK 2240 and TK 2242 (see Table I). The remaining activity of the potassium uptake system is proportional to the extracellular potassium concentration [1] and too low to allow this strain to grow under our experimental conditions (with 1 mM potassium as limiting substrate).

Discussion

The steady-state dry weight of the two *E. coli* strains TK 2240 and TK 2242, grown under potassium-limited conditions, showed a decrease with increasing growth rate. The same behaviour was seen before [2] with two other *E. coli* strains, FRAG-1 (the parent) and FRAG-5 (a mutant lacking the high-affinity potassium uptake system) that were cultured under the same experimental conditions.

The possibility of futile cycling of potassium ions via the (catalytic) mechanism proposed by us is not yet firmly established. It could be argued that the difference in ATP turnover between FRAG-1 and FRAG-5 [2] was due to the fact that FRAG-1, having both low- and high-affinity potassium uptake systems, could generate a much steeper gradient than FRAG-5. Because of this steeper gradient, FRAG-1 would have a larger passive leak and, therefore, have to invest more energy to maintain the gradient. Therefore, we wanted to grow a mutant in the chemostat that totally lacked the Trk system, but possessed the Kdp system. The Trk system is very complex [4]. Thus far, no mutants have been found that lack this low-affinity system completely. However, a mutant existed that had only a very low overall uptake rate via the Trk system [3].

This strain, TK 2240, was tested in the chemostat under potassium-limited conditions which induce the high-affinity potassium uptake system (Kdp) [6,7]. If the higher ATP turnover in FRAG-1 compared to FRAG-5 was due to the higher energy requirement of the Kdp system, one would expect that strain TK 2240 would show the same ATP turnover as FRAG-1 (cf. Ref. 2). However, it was found that the ATP turnover for this mutant, as calculated from its products, was less than that for FRAG-1, but still higher than that for FRAG-5.

The question arose whether this increased ATP turnover for TK 2240 as compared to that for FRAG-5 was related to the low K_m (0.002 mM) of the Kdp system. This was tested with strain TK 2242. *E. coli* TK 2242 is a mutant that has the same low activity of the low-affinity system as TK 2240. It differs from TK 2240, however, in that the affinity of the mutated Kdp system for potassium is 100-fold lower (K_m = 0.3–0.5 mM) (personal communication of Dr. E.P. Bakker) than for the wild-type Kdp system (K_m = 0.002 mM) [1]. Thus, the K_m of the Kdp (high-affinity) uptake system in TK 2242 is similar to that of the wild-type Trk (low-affinity) system. Because of this lower affinity, one could speculate that TK 2242 would show a similar ATP turnover as *E. coli* FRAG-5, a strain totally lacking the high-affinity system (cf. Ref. 2), because both strains have a potassium uptake system with comparable kinetic parameters (cf. Table I). On the other hand, the K^+/ATP stoichiometry of the mutated Kdp system is probably not changed. From Fig. 2 it is clear that the ATP production rate for strain TK 2242 is the same as for TK 2240. This means that growth under potassium-limited conditions is independent of the kinetic parameters of the potassium uptake system. The difference in ATP turnover between FRAG-5 and both TK 2240 and TK 2242 must be due to the higher ATP/K^+ ratio of the Kdp system than that of the Trk system.

Both TK 2240 and TK 2242 are able to take up potassium via the high-affinity system. Although the K_m of the Kdp system in TK 2242 was changed (100-fold higher) in comparison to TK 2240, both strains were still able to scavenge traces of potassium from the extracellular medium. The extracellular potassium concentration as measured was the same as was found for FRAG-1 before [2]. At first sight it seems not possible that TK 2242 would be able to maintain such a low extracellular potassium concentration with the given kinetic

parameters (Table I). However, we should keep in mind that the kinetic data reported in the literature, were obtained with cells that were grown in batch culture. It is possible that under our K^+-limited conditions in the chemostat, more Kdp is present in the cells. In any case, the strains were continuously tested for their properties on agar plates (K-0.3 and K-115 plates) (personal communication of Dr. E.P. Bakker) and no indications for a return to the wild-type were found. This indicates, together with the results of Fig. 2 and the ATP turnover of FRAG-1 and FRAG-5 [2], that the potassium efflux is decreased in the strains with a decreased Trk uptake system.

The results obtained with the different strains of *E. coli* mutated in one or both of the potassium uptake systems can be explained in terms of futile cycling of potassium ions, as proposed before [2]. The yield on potassium depends on the capacity to maintain a certain intracellular concentration of this ion. If a facilitated leakage pathway is present, the energetic cost of this process will be higher.

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References

1 Rhoads, D.B., Waters, F.B. and Epstein, W. (1976) J. Gen. Physiol. 67, 325–341.
2 Mulder, M.M., Teixeira de Mattos, M.J., Postma, P.W. and Van Dam, K. (1986) Biochim. Biophys. Acta 851, 223–228.
3 Rhoads, D.B., Laimins, L. and Epstein, W. (1978) J. Bacteriol. 135, 445–452.
4 Epstein, W. and Laimins, L. (1980) TIBS 5, 21–23.
5 Epstein, W., Whitelaw, V. and Hesse, J. (1978) J. Biol. Chem. 253, 6666–6668.
6 Wieczorek, L. and Altendorf, K. (1979) FEBS Lett. 98, 233–236.
7 Epstein, W. (1985) Current Topics in Membranes and Transport 23, 153–175.