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REGULATION OF THE INTRACELLULAR POTASSIUM CONCENTRATION IN *ESCHERICHIA COLI* B 525

U. ZIMMERMANN, G. PILWAT and TH. GÜNTHER[a]

Institute for Physical Chemistry, Nuclear Research Center, Jülich and [a]*Central Institute for Biochemistry and Biophysics, Free University Berlin, Berlin (Germany)*

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SUMMARY

The mutant *Escherichia coli* B 525 requires histidine, leucine and methionine and an elevated extracellular K^+ concentration for growth, and is unable to retain K^+ tightly inside the cells when incubated in media supplemented with glucose, arabinose, galactose or lactose as the sole energy and carbon source. The loss of K^+ from the cells of B 525 can be prevented by adding histidine and leucine, which react specifically and only in combination. In media supplemented with glycerol as the substrate, with glucose and NH_4^+, or with glucose under anaerobic conditions, a stationary level of K^+ inside the cells can be obtained without the addition of histidine–leucine.

On the addition of ribose to glycerol-adapted cells of B 525 preincubated in glycerol media, the intracellular K^+ decreased immediately and markedly. This decrease can be overcome by the addition of histidine–leucine.

INTRODUCTION

The accumulation of K^+ and the reciprocal exclusion of Na^+ from living cells are dependent on metabolic energy and belong to the fundamental phenomena of life.

So far, six K^+-deficient mutants of *Escherichia coli* have been described in the literature. Starting with the *E. coli* K 12 strain as the parent, Epstein and Kim[1] isolated a single type of mutant called Kdp, for K^+-dependent. This mutation resulted in a modest increase in the requirement of K^+ for growth. Using a strain with a Kdp mutation as the parent, double mutants were obtained combining the Kdp mutation with a mutation in one or more of five other loci, called trk (A–E), for transport of K. The mutations in these loci caused an alteration of K^+ transport. The K^+-deficient mutant *E. coli* B 525 investigated in this paper was isolated by Lubin and Kessel[2] in 1960 from B 163, which requires histidine, leucine and methionine. According to Epstein and Kim[1], B 525 has a defect in location consistent with trkC and is defective in K^+ retention in agreement with findings for the trkC mutants.

The mutant *E. coli* B 525 requires approximately 10^{-3} moles/l K^+ for growth, whereas the precursor B 163 grows well at K^+ concentrations below 10^{-6} moles/l[3].

Cells of B 525 show a considerably greater dependence of the intracellular K^+ concentration on the extracellular K^+ concentration than does B 163 (ref. 4).

Moreover, the K^+ accumulated within a few minutes in the cells of B 525 deprived of the three required amino acids is not constant[5]. With an external K^+ concentration of 12 mmoles/l and pH 7 for the incubation medium, the accumulated K^+ drops in the course of 4 h from about 180 mmoles/l to about 60 mmoles/l. In comparision, the loss of K^+ observed in wild-type B 163 under identical experimental conditions was only small and the K^+ level became constant after 1–2 h.

A stabilization of the K^+ concentration in B 525 can be obtained in media supplemented with histidine and leucine, in combination only, or with NH_4^+. The time course of the K^+ concentration under these conditions is similar to the case of B 163 in the absence of the required amino acids, and can be divided into three stages: Stage I, a period of rapid uptake (5 min), Stage II, a period during which the intracellular K^+ concentration decreases slowly (1–2 h), and Stage III, a period of a stable steady state.

Methionine, which like histidine and leucine is required by both strains for growth, or combinations of methionine with one of the two amino acids indicated, showed no significant effect[5].

In continuance of the previous experiments an attempt was made in this paper to explain the K^+ decrease in B 525 during Stage III and the effect of histidine–leucine and NH_4^+.

METHODS

E. coli, strains B 525 and B 163, which require histidine, leucine and methionine for growth, were used throughout the experiments. The methods of growth and harvesting of the organisms, as well as the methods used for determining intracellular cation concentrations, have previously been described in detail[5].

Briefly, the organisms were transferred directly from the stock plates into a growth medium of following composition: KCl, 30 mM; $MgCl_2$, 1 mM; Na_2HPO_4, 90 mM; NaH_2PO_4, 30 mM; $(NH_4)_2SO_4$, 15 mM; histidine, methionine, and leucine, 100 mg each; and lactose, 5 g. The mutants were tested in "potassium-free" growth medium for revertants in each experiment, since B 525 is unable, in contrast to B 163 cells, to concentrate and utilize for growth the small amounts of K^+ contaminating the constituents of "potassium-free" medium. The bacteria were grown at 37 °C, and were harvested in the stationary phase after 16 h of growth.

The basic composition of the incubation medium was: Tris, 110 mM; HCl, 90 mM; $MgSO_4$, 1 mM; KCl, 12 mM; Na_2HPO_4, 40 mM; NaH_2PO_4, 20 mM. The pH of the incubation medium was 7.2. The desired experimental conditions were obtained by the addition of amino acids and various substrates. The experiments were carried out at 37 °C by passing O_2–CO_2 (95:5, v/v) through the suspension. Aliquots were removed at various times, centrifuged, weighed, dried overnight at 90 °C in a vacuum drying oven (1 Torr) and reweighed. After ashing in a Tracerlab low temperature asher, the potassium determinations were made using a Zeiss PMQ II flame photometer. The intracellular K^+ concentration was calculated using the equation of Schultz and Solomon[6]. The determination of the extracellular space has previously been described[5]. The glucose consumption of the bacteria was measured by determi-

nation of the time course of the glucose concentration of the suspension by the hexokinase method using the Biochemica Test Combination of Boehringer Mannheim GmbH. The glucose 6-phosphate concentration in the cells was determined according to the method of Lowry *et al.*[7].

Samples were taken at various times from the suspension, and were filtered through 0.45 μm Millipore filters. The filters were put into centrifuge tubes, frozen in liquid air and broken into small pieces. Cold 0.3 M $HClO_4$, containing 1 mmole/l EDTA, was added to the tubes, the tubes were thoroughly mixed, and centrifuged at $10000 \times g$. The supernatant fluid was neutralized with solid K_2CO_3 and filtered through 0.45-μm Millipore filters.

Glucose 6-phosphate was determined by measurement at 340 nm of the NADPH which is formed by the dehydrogenation of glucose 6-phosphate with glucose 6-phosphate dehydrogenase.

For the radiochromatographic analysis of the perchloric acid extract of B 525 and B 163 cells after uptake of [U-^{14}C]ribose (3.0 Ci/mole, Amersham) and [U-^{14}C]-xylose (3.0 Ci/mole, Amersham) the method of thin-layer chromatography described by Waring and Ziporin[8] was used.

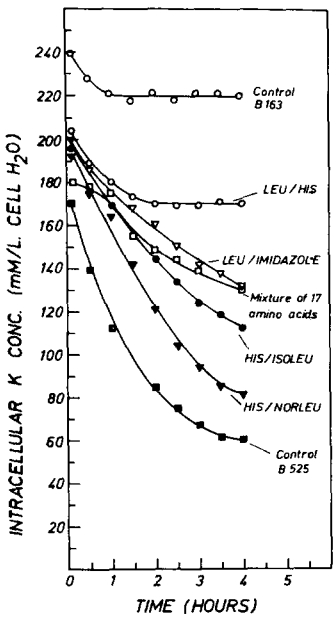
The plates coated with cellulose powder MN 300 (Macherey, Nagel and Co. Düren, Germany) were developed two-dimensionally. Phase I, the water-poor phase, was a mixture of 60 ml *tert*-amyl alcohol, redistilled at 101.8 °C, 30 ml water and 2 g *p*-toluenesulfonic acid. Phase II was a mixture of 66 ml isobutyric acid, redistilled at 154.4 °C, 1 ml concentrated ammonium hydroxide and 33 ml water. The distribution of the radioactivity on the plates was measured using the thin-layer scanner II of Berthold–Frieseke Vertriebs-GmbH.

RESULTS

Effects of amino acids on K^+ retention

To decide whether the stabilization of the K^+ level in B 525 is a specific effect of histidine and leucine, both the individual effects of several single amino acids and of combinations of various amino acids with histidine on the intracellular K^+ concentration were examined. In an additional experiment, a mixture of 17 natural L-amino acids, omitting histidine, leucine and methionine, was tested. The bacteria were incubated in the basal salt solution supplemented with 1% glucose. The amino acid to be tested was added at a final concentration of 100 mg/l. The concentration of each amino acid of the mixture of 17 amino acids was only 20 mg/l in order to obtain a total amino acid concentration of the same order as that in other experiments. According to Fig. 1, only the combination of histidine and leucine prevents the net loss of K^+. However, in media supplemented with other amino acids the decrease of K^+ is slower than in the unsupplemented medium. Nor was it possible to stabilize the asymmetric K^+ distribution between the B 525 cells and the external medium by replacing histidine with imidazole. In this case, however, the loss of K^+ was strongly retarded so that the stabilizing effect of histidine on the potassium concentration in the cells may, in part, be dependent on its imidazole group[5].

As mentioned above, NH_4^+ also have a stabilizing effect on the K^+ concentration in B 525 cells, but only at substantially higher concentrations (Fig. 2). The optimum NH_4^+ concentration is about 20 mmoles/l in the incubation medium as



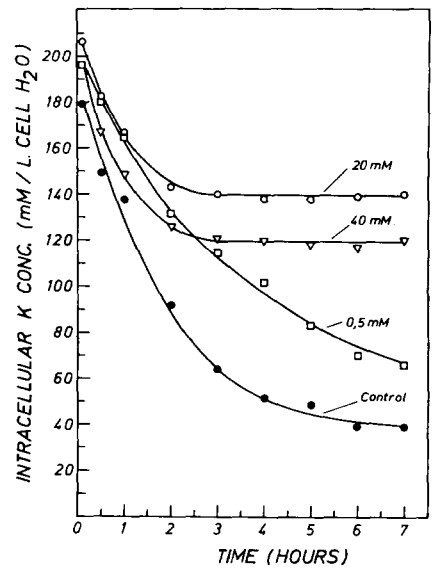


Fig. 1. Effects of amino acids (100 mg each) on the time course of the intracellular K^+ concentration of *E. coli* B 525 at 12 mmoles/l extracellular K^+. Cells were grown overnight in media supplemented with 30 mmoles/l K^+, histidine, leucine, methionine (100 mg/l each) and 1% lactose, resuspended in media containing 1% glucose and 12 mmoles/l K^+, and divided into six aliquots, to which leucine–histidine (○—○), leucine–imidazole (▽—▽), histidine–isoleucine (●—●), histidine–norleucine (▼—▼), and a mixture of 17 amino acids (□—□) were added. The amino acids had the L-configuration. The control suspensions of B 525 (■—■) and B 163 (○—○) did not contain amino acids. The curve for the effect of valine–histidine (not shown) coincide with the curve for histidine–norleucine. The data points are average values from four different sets of measurements.

Fig. 2. Effects of NH_4^+ concentration on the time course of the intracellular K^+ concentration of *E. coli* B 525 at 12 mmoles/l extracellular K^+. Growth and procedure as in Fig. 1 except that, instead of amino acids, NH_4^+ was added at concentrations of 0.5 mM (□—□), 20 mM (○—○), and 40 mM (▽—▽). The control suspension (●—●) did not contain NH_4^+. The data points are average values from four different sets of measurements.

against 100 mg/l (approx. 1 mmole/l) for the two amino acids histidine and leucine (see also ref. 5).

The intracellular K^+ concentration is not further raised by increased histidine and leucine concentrations up to 100 mmoles/l, whereas an increase of NH_4^+ concentration above 20 mmoles/l, results in a decrease in the stationary intracellular K^+ concentration. Presumably, this decrease may be due to a superimposed, competitive inhibition of K^+ transport caused by NH_4^+.

When histidine–leucine or NH_4^+ are restored after 3 h to the K^+-depleted cells in the unsupplemented media, an accumulation of K^+ occurs again, reaching levels which closely resemble those of cells which were incubated in media supplemented with histidine and leucine from the beginning of incubation. This indicates that the loss of K^+ is not irreversible, which is in agreement with previous findings[5].

According to Table I, the influence of the amino acid combination histidine–

leucine or of NH_4^+ on the K^+ level in the B 525 cells cannot even be ascribed to an increase in metabolism resulting from these compounds. It is true that the oxygen and glucose consumption are raised. However, this effect is non-specific since other amino acids with no effect on the K^+ concentration also cause an approximately equal increase in the respiration rate and glucose consumption. Since respiration and glucose consumption, both in the presence and absence of those compounds, are constant for more than 4 h, the loss of K^+ cannot be explained by a decrease in metabolic degradation.

TABLE I

OXYGEN AND GLUCOSE CONSUMPTION OF *E. COLI* B 525 AND B 163

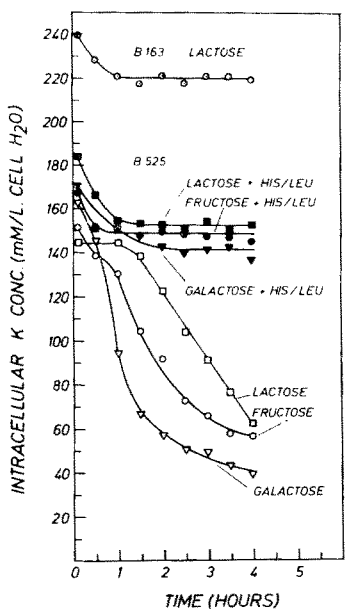
The concentration of amino acids and imidazole was 100 mg/l. The ammonium chloride concentration was 20 mmoles/l. Each medium contained 1% glucose. The values are mean averages from two different sets of measurements.

Compounds	*Oxygen consumption (µl O_2 per mg wet weight per h)*		*Glucose consumption (µg glucose per mg wet weight per h)*	
	B 525	*B 163*	*B 525*	*B 163*
Control	37.9	33.3	103.7	83.3
Histidine–leucine	58.3	56.2	187.7	106.1
Leucine–imidazole	55.4	55.6	163.5	97.5
Histidine–isoleucine	55.8	55.6	186.6	89.0
Histidine–valine	53.0	55.6	172.1	93.3
Histidine–norleucine	54.8	54.8	148.5	95.5
Histidine–aspartic acid	58.6	55.9	163.4	98.6
Histidine–glutamic acid	57.2	56.1	164.4	93.4
Ammonium chloride	59.5	55.7	156.0	101.1

Effects of substrates on the K^+ retention

In another set of experiments we investigated the extent to which the K^+ level in B 525 cells is dependent on the carbon and energy source in the medium. In media supplemented with 1% lactose, fructose, galactose or arabinose as the sole source of energy, a similar decline of the level of K^+ in lactose-grown B 525 cells was observed (Fig. 3). In contrast, the intracellular K^+ level of B 163 cells was not influenced by these sugars. However, in glycerol-grown B 525 cells incubated in the basal salt medium supplemented with glycerol as the carbon and energy source, a stationary intracellular potassium level of about 80 mmoles/l was established after 5 min or, more frequently, after a short period of 30 min (Fig. 4). An addition of 100 mg/l histidine and leucine resulted only in a further uptake of 10 mmoles/l K^+. The K^+ concentration is insensitive to changes of the glycerol concentration within the range of 0.4% to 10% glycerol. In glycerol-unadapted cells of the mutant, a K^+ level of 40 mmoles/l was measured (Fig. 4).

In contrast, glycerol-unadapted and glycerol-adapted B 163 cells accumulate K^+ in glycerol media to a level of 180–200 mmoles/l as found in lactose-grown bacteria incubated in media supplemented with 1% glucose. The K^+ level is immediately constant.



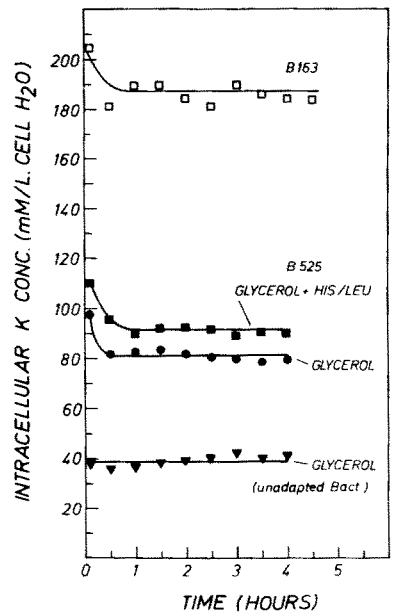
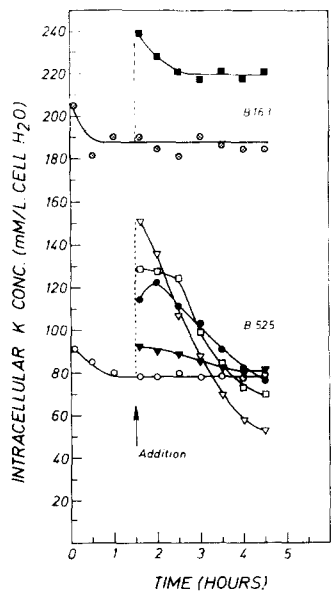


Fig. 3. Effects of galactose, fructose and lactose on the intracellular K^+ concentration of *E. coli* B 525 at 12 mM extracellular K^+. Lactose-grown cells (for details of growth see Fig. 1) were resuspended in media supplemented with 12 mM K^+, 100 mg/l histidine–leucine, and with 1% galactose (▼—▼), 1% fructose (●—●) and 1% lactose (■—■), respectively. Control suspensions of B 525 with 1% galactose (▽—▽), 1% fructose (○—○) and 1% lactose (□—□), respectively, and of B 163 with 1% lactose (⊗—⊗) did not receive the two amino acids. The curves of the time-dependence of the intracellular K^+ concentration in B 163 after the addition of galactose and fructose, respectively, not shown in the figure, coincide with the curve obtained in the presence of lactose. The data points are average values from four different sets of measurements.

Fig. 4. Effects of glycerol on the intracellular potassium concentration in B 525 at 12 mM extracellular K^+. Cells were grown on 1% glycerol, 30 mM K^+ and histidine, leucine, methionine (100 mg/l each); incubation in media containing 1% glycerol and 12 mM K^+ (●—●). The suspension was divided into two aliquots; to one of which 100 mg/l histidine and leucine (■—■) were added. In a parallel experiment lactose-grown cells of B 525 were suspended in media containing 1% glycerol and 100 mg/l histidine and leucine (▼—▼). Control suspensions of glycerol-adapted and glycerol-unadapted B 163 cells (□—□) did not contain histidine and leucine. The data points are average values from four different sets of measurements.

The addition of glucose, fructose, galactose, lactose or arabinose to glycerol-adapted B 525 cells, preincubated for 1.5 h in glycerol-media, rapidly stimulated the K^+ uptake to the same level as measured for lactose-grown bacteria incubated in media supplemented with the respective sugars (Fig. 5). In the following 3 h the intracellular K^+ level decreases as usual and reaches, for instance, a value of about 50 mmoles/l with glucose. The K^+ drop can be overcome by adding histidine–leucine to the glycerol medium. With the addition of 2-deoxyglucose and methyl-α-glucose, *i.e.* glucose analogues and nonmetabolizable sugars, to glycerol-adapted cells preincubated in glycerol-media, the K^+ level decreased in a manner scarcely dependent on the concentration of these sugars. On the other hand, ribose, which is only poorly utilized for growth by B 525 cells, under these conditions caused a marked reduction



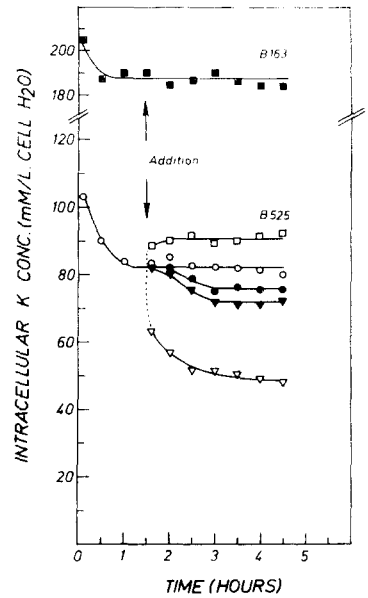


Fig. 5. Glycerol-adapted B 525 cells (growth details as in Fig. 4) were preincubated in media containing 1% glycerol and 12 mM K^+ (○—○). Division into four aliquots and addition of 1% arabinose (▼—▼), 1% glucose (▽—▽), 1% fructose (□—□) and 1% galactose (●—●), respectively, at the time indicated by the arrow, resulted in an immediate increase and a similar subsequent decrease of the intracellular K^+ levels as found in lactose-grown cells in the absence of histidine and leucine (see Figs 1 and 2). The addition of the sugars to control suspensions of glycerol-adapted and glycerol-preincubated B 163 cells (⊗—⊗) resulted in a modest increase of the intracellular K^+ only in the case of 1% glucose (■—■), whereas galactose and arabinose did not alter the K^+ level established in glycerol-media. The data points are average values from four different sets of measurements.

Fig. 6. Effects of ribose, 2-deoxyglucose and methyl-α-glucoside on the intracellular K^+ level of glycerol-adapted *E. coli* B 525 cells. Growth and preincubation as in Fig. 5. After preincubation for 1.5 h (○—○) the suspension was divided into four aliquots and 1% ribose (▽—▽), 1% 2-deoxyglucose (▼—▼) and 1% methyl-α-glucoside (●—●), respectively, were added. The addition of ribose resulted in a significant decrease of the intracellular K^+ concentration. This decrease can be overcome by the addition of 100 mg/l histidine and leucine (□—□). The addition of the sugars to control suspensions of B 163 cells (■—■) treated in the same manner did not alter the K^+ level. The data points are average values from four different sets of measurements.

of the K^+ concentration to about 50 mmoles/l at a concentration of 1% (Fig. 6). This loss is dependent on the ribose concentration. It was greatest with 100 mmoles/l ribose, and there was not more than 10 mmoles/l K^+ in the presence of 1 mmole/l ribose. The addition of histidine–leucine reverses the effect of ribose, suggesting that these sugars or their derivatives have a common site of action on K^+ transport.

The sugars tested above, in the case of B 163 do not interfere with the K^+ level of glycerol-adapted cells incubated under the same conditions. This indicates that the effects observed in B 525 must be related to its mutation.

Preliminary radiochromatographic analyses of the perchloric acid extract of glycerol-adapted cells incubated in glycerol medium after the uptake of [U-^{14}C] ribose indicate only small amounts of free ribose. The major part of the radioactivity

(about 70%), was found in a substance which was probably glucose 1-phosphate as indicated by the addition of labelled glucose 1-phosphate to the inactive cell extracts of B 525 and subsequent chromatographic developement. Five other spots on the radiochromotogram have not yet been identified. Therefore, it cannot be decided at the present time if glucose 1-phosphate is responsible for the intracellular decrease of K^+ caused by ribose.

In this context, it should be mentioned that in the cell extracts of B 163 most of the radioactivity is found in free ribose after the uptake of labelled ribose during the first hour (90%). During the following 2 h the same radioactive pattern was detected in chromatograms of cell extracts of B 163 as found in those of the mutant immediately after the beginning of uptake. This result, and the fact that the rate of uptake of ribose is much slower in comparison with the mutant indicate a further defect in B 525 caused by the mutation. About this, we will report in a forthcoming paper.

1% sucrose, raffinose, and xylose do not show any reaction under the same experimental conditions. The absence of such an effect caused by sucrose and raffinose on the intracellular K^+ concentration may be explained by their restricted permeation through the cell membrane. Xylose, however, does permeate into the cells, as measured by uptake of [U-^{14}C] xylose. Most of the radioactivity (90%) in the cell extracts was detected by radiochromatography in the accumulated xylose, just as in the wild type after the uptake of [^{14}C]xylose. Under anaerobic conditions, *i.e.* with the passage of nitrogen through the suspension, the time course of the K^+ concentration in the lactose-grown B 525 cells usually observed under aerobic conditions in media supplemented with glucose (see control of Fig. 1) is altered (Fig. 7). Although the medium did not contain histidine and leucine, a stationary K^+ content in the cells was established after 2 h. This K^+ concentration is kept constant in B 525 during a further period of 2 h and more. In Stage III the stationary level is in the range of 120 mmoles/l and, thus, corresponds approximately to the value found in media with 40 mmoles/l NH_4^+ under aerobic conditions. By adding histidine and leucine, the intracellular K^+ concentration is raised and attains a value of about 160 mmoles/l.

It is well known that the concentration of hexosephosphates and, perhaps, of the hexoses themselves inside the cells, is decreased under anaerobiosis by allosteric activation of phosphofructokinase[9]. Therefore, one might postulate that glucose 6-phosphate or glucose causes the instability of the K^+ level in the cells of B 525. The effect of NH_4^+ can be interpreted on the same basis, since NH_4^+ will increase aerobic glycolysis by a specific activation of the phosphofructokinase[10,11]. In consequence, the glucose 6-phosphate would be reduced[12] (see below).

The optimum concentration of NH_4^+ for the activation of phosphofructokinase is between 20 mmoles/l and 50 mmoles/l and, therefore, corresponds well to the concentration necessary to stabilize the intracellular K^+ concentration. Since both these experiments, and some experimental data from the studies of Harold and Baarda[13] on *Streptococcus faecalis* lend support to a regulatory role of glucose 6-phosphate on the K^+ level of B 525, we determined the glucose 6-phosphate level in B 525 and B 163 by enzymatic methods, incubating the bacteria in media supplemented with 1% glucose and histidine–leucine, NH_4^+, glutamic acid and aspartic acid, respectively.

The glucose 6-phosphate level is constant over a period of 3 h. NH_4^+ lowers the glucose 6-phosphate level significantly (about 30%) in comparison with the con-

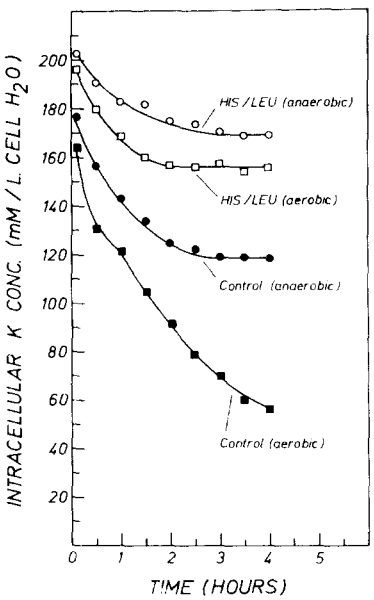


Fig. 7. Influence of anaerobic conditions on the intracellular K^+ level of *E. coli* B 525 grown in media containing 30 mM K^+, 1% lactose, and histidine, leucine and methionine (100 mg/l each). The cells were resuspended in media containing 12 mM K^+ and 1% glucose, and nitrogen was passed through the suspension (●—●) which was divided into two aliquots, to one of which 100 mg/l histidine and leucine (○—○) were added. In parallel control experiments carbogen was passed through the suspension in the absence (■—■) and presence (□—□) of histidine and leucine. The data points are average values from four different sets of measurements.

trol, as expected. There is also a slight fall of the glucose 6-phosphate level (about 10%) after the addition of histidine–leucine, but the same is true for other amino acids having no stabilizing effect on the K^+ accumulation in B 525. Furthermore, the same effects are obtained in B 163. Therefore, it can be concluded that the different behaviour of the mutant and the wild type with regard to the K^+ retention does not arise from a different level of glucose 6-phosphate inside the cells.

DISCUSSION

At the present time we have little information concerning either the nature of the effect of histidine and leucine and of hexoses and pentoses (arabinose and ribose) on K^+ retention in *E. coli* B 525, nor do we know why the other tested amino acids and sugars appear to play no role in this regard. Clearly, we are only at the earliest stages in reaching a full understanding in this complex phenomenon. Therefore, we can only speculate at the moment that these two amino acids somehow influence the interactions between sugar transport, catabolism, and the K^+ transport. It is known that amino acids play a specific role in catabolite repression[14] and in phenomena of stringent control of transport and of many biosynthetic reactions[15]. Both phenomena may be partly responsible for our results. According to the catabolite repression hypothesis, the synthesis of a sensitive enzyme should be inhibited when a sugar is metabolized so rapidly that the intracellular level of catabolites

exceeds the requirement for the biosynthetic reactions. Under unbalanced conditions, as in our experiments in which the auxotrophic mutant B 525 is deprived of the required amino acids, a much stronger repression of enzyme synthesis depending on the concentration and on the nature of the carbon source should be expected than that observed in normally growing cells[16]. Due to our results we must assume a repression of the synthesis of a sensitive enzyme involved in K^+ transport and we must furthermore postulate that the repression of this enzyme is altered by the mutation since the same unbalanced conditions are true for the precursor B 163, *i.e.* the enzyme synthesis in B 525 must be much more severe repressed than in B 163. This repression can be overcome specifically by the addition of histidine and leucine. If this hypothesis is true, the K^+ level inside the cells would be regulated by the energy supply of the carbon source and by repression of an enzyme of the K^+ transport by means of catabolites derived from the degradation of the carbon source.

The effector of catabolite repression might be a phosphate ester which is easily derived from glucose, such as glucose 1-phosphate; this seems possible considering the ribose experiment. It also appears possible that there are some relationships to the phosphoenolpyruvate: sugar phosphotransferase system (PTS) which is associated with the cell membrane and has a central role in the transport of sugars, in the energy coupling of transport, in the induction of catabolic enzyme systems, and in the transient and catabolite repression systems[17,18].

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