Inhibitory Effect of Li⁺ on Cell Growth and Pyruvate Kinase Activity of Escherichia coli

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Li⁺ inhibited growth of Escherichia coli when glucose, galactose, fructose, or glycerol was added as the sole source of carbon. Growth inhibition was not observed when lactate or a mixture of amino acids was used as the carbon source. A mutant possessing elevated activity of Li⁺ extrusion was not inhibited by Li⁺. These results suggested that intracellular Li⁺ inhibited the glycolytic pathway, most likely triose metabolism, without affecting gluconeogenesis. We also found that pyruvate kinase I was inhibited by Li⁺.

We have previously reported that Li⁺ strongly inhibits the growth of Escherichia coli when melibiose is added as the sole source of carbon (12, 16). Such strong inhibition has not been observed in cells possessing the lactose transport system. Melibiose transport via the melibiose transport system is inhibited by Li⁺ (14, 16). However, one problem remains unsolved. There is a difference in extent between the effect of Li⁺ on the growth of cells and the effect on melibiose transport. The growth of E. coli on melibiose is completely inhibited by Li⁺, but the inhibition of melibiose transport is only about 80%. Thus, another process involved in melibiose metabolism should be considered to explain the Li⁺ effect on the growth.

E. coli W3133-2 (7) and W3133-2S (12), both of which are K-12 strains lacking the lactose transport system, were used in the present study. Strain W3133-2S is a mutant derived from W3133-2 and possesses an elevated activity of Li⁺ extrusion (12). Cells were grown in a minimal salts medium (15) (Na⁺ salts were replaced with K⁺ salts) supplemented with each carbon source at 37°C under aerobic conditions.

Pyruvate kinase I was partially purified, and activity was measured as described before (19). NADH oxidase activity in the partially purified preparations was minimal. Protein was determined by the method of Lowry et al. (8).

Growth of E. coli cells was strongly inhibited by Li⁺ when melibiose was added to the medium as the sole source of carbon (Fig. 1A) (12, 16). It has been shown that Li⁺ inhibits melibiose uptake via the melibiose transport system (14, 16). When methyl-α-galactoside, another substrate for the melibiose carrier, was added as the sole carbon source, growth of the cells was inhibited by Li⁺ to some extent (Fig. 1B). This was an unexpected observation because Li⁺ does not inhibit the transport of methyl-α-galactoside (17). On the contrary, Li⁺ stimulates methyl-α-galactoside transport by the cotransport mechanism (17). The lithium ion actually stimulates melibiose transport in Salmonella typhimurium cells (11). Growth of the cells, however, was inhibited by Li⁺ to some extent when melibiose was the sole source of carbon. These results suggested that the growth inhibition caused by Li⁺ was partly caused by the inhibitory effect of Li⁺ on a metabolic pathway(s).

To clarify the effect of Li⁺ on metabolism, we tested the effect of Li⁺ on cell growth with various carbon sources. When glucose, galactose, fructose, or glycerol was the carbon source, 30 to 100 mM LiCl inhibited the growth rate about 50 to 70% (Fig. 2A). No significant growth inhibition was caused by KCl or NaCl in this concentration range (data not shown). Almost no growth inhibition was caused by Li⁺ when lactate or tryptone (amino acid mixture) was the carbon source (Fig. 2A). Thus, only when carbon sources metabolized via the glycolytic pathway were added was growth inhibited by Li⁺. It seems that Li⁺ does not have a significant effect on other vital pathways, including gluconeogenesis, because Li⁺ did not inhibit cell growth on lactate or the mixture of amino acids. Furthermore, we suggest that among the glycolytic pathways, an enzyme involved in triose metabolism was the target of the Li⁺ effect because utilization of glycerol was also inhibited by Li⁺.

If intracellular Li⁺ is poisonous, then it would be interesting to test whether similar growth inhibition by Li⁺ occurs with a mutant possessing an elevated activity of Li⁺ extrusion. If intracellular Li⁺ has an inhibitory effect on glycolysis, such a mutant would be able to grow normally in the presence of Li⁺. A concentration of 30 to 100 mM LiCl did not cause detectable growth inhibition of the mutant even when glucose, galactose, fructose, or glycerol was the carbon source (Fig. 2B). This indicates that the effect of ionic strength on growth at this concentration range of salts was not significant. Therefore, it is very likely that Li⁺ enters the cells by diffusion and that with an external concentration of 100 mM, sufficient Li⁺ enters the wild-type cells to inhibit metabolism.

We investigated which enzyme in the glycolytic pathway was inhibited by Li⁺. As mentioned above, we had two clues for this question. First, an enzyme involved in triose metabolism should be inhibited. Second, it should be the enzyme involved in glycolysis but not in gluconeogenesis. Five enzymes participate in triose metabolism, namely, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, phosphoglyceromutase, enolase, and pyruvate kinase (6). Among the processes catalyzed by these enzymes, conversion of phosphoenolpyruvate to pyruvate (catalyzed by pyruvate kinase) is the sole irreversible process (6). In gluconeogenesis, phosphoenolpyruvate is synthesized from pyruvate via oxaloacetate without the involvement of pyruvate kinase (6). Thus, it was deduced that pyruvate kinase might be the enzyme inhibited by Li⁺.

Two types of pyruvate kinase (I and II) are known in E. coli (9). The type I enzyme, which is activated by fructose-1,6-diphosphate, also occurs in a diversity of other organisms (3, 19). Pyruvate kinase I has been suggested to be
physiologically important during glycolysis (9, 19). The effect of Li⁺ and other monovalent cations on the activity of pyruvate kinase I was tested. Li⁺ (50 mM) inhibited pyruvate kinase I activity rather strongly (Table 1). A similar strong inhibition of the pyruvate kinase I prepared from strain W3133-2S (a mutant whose growth was not inhibited by Li⁺) was also observed (Table 1). Sodium and choline ions also inhibited the enzyme activity to some extent. Other monovalent cations such as K⁺, NH₄⁺, and Rb⁺ had no significant effect at 50 mM. It should be noted that none of these cations inhibited lactate dehydrogenase activity, which was involved in the pyruvate kinase assay (data not shown).

K⁺ at a concentration of 10 mM stimulated pyruvate kinase activity about 30% (data not shown). A very strong inhibition of the enzyme activity was caused by Tl⁺. The thallium ion caused complete growth inhibition of E. coli when added to the medium (data not shown). Pyruvate kinase I, however, seems not to be a sole target of the inhibition caused by Tl⁺ because Tl⁺ inhibited cell growth irrespective of the carbon source (data not shown).

Although pyruvate kinase I was inhibited by Li⁺, pyruvate kinase II was not significantly affected by Li⁺ (data not shown). Pyruvate kinase II is defined as AMP-activated pyruvate kinase in the absence of fructose-1,6-diphosphate (19). The physiological significance of pyruvate kinase II has not been clarified.

Figure 3 shows the effect of Li⁺ concentration on the activity of pyruvate kinase I. The concentration of Li⁺ giving half-maximal inhibition was 20 mM. The concentration range of Li⁺ giving strong inhibition of pyruvate kinase I activity was above ca. 30 mM, which was consistent with the effect on cell growth. Since pyruvate kinase is a regulatory enzyme in glycolysis (6), inhibition of this enzyme slows down glycolysis. Thus, when a glycolytic substrate is the sole source of carbon, growth of cells is slowed. At a concentration of 100 mM, Li⁺ inhibited the activity of the isolated enzyme almost completely, whereas the growth of cells exposed to this concentration of Li⁺ was inhibited by about 75%. This discrepancy could be caused by the following. (i) Pyruvate kinase II could function in the presence of Li⁺. (ii) The intracellular Li⁺ concentration would be lower than that of the medium because Li⁺ is extruded via the Li⁺/H⁺ antiporter (13). (iii) Pyruvate kinase I may not be an entirely rate-limiting enzyme in glycolysis. (iv) Glycolysis may not be completely rate limiting for cell growth even when a glycolytic substrate is the sole carbon source.

The effect of Li⁺ on the kinetic parameters of pyruvate kinase I was then tested. The lithium ion (at 50 mM) lowered the $V_{max}$ of pyruvate kinase I (from 0.89 to 0.15 μmol of NADH utilized per min per mg of protein) without affecting the $K_m$ (0.67 mM) (data not shown).

The effect of monovalent cations on the pyruvate kinase I has been investigated with several organisms. K⁺ or NH₄⁺ stimulates the enzyme (1, 2, 4, 10, 18). The mechanism of activation of pyruvate kinase by K⁺ and NH₄⁺ has not been clarified. It has been reported, however, that K⁺ facilitates the binding of phosphoenolpyruvate to the enzyme (18). It has also been reported that the stimulation of muscle pyruvate kinase by K⁺ is reversed by high concentrations of Li⁺.

![FIG. 2. Effect of Li⁺ concentration on the growth rate of E. coli. Cells were grown on 40 mM lactic acid (Δ), 1% tryptone (■), 20 mM glucose (□), 20 mM galactose (●), 20 mM fructose (△), or 40 mM glycerol (▲) in the presence of various concentrations of LiCl. The growth rate was calculated as the doublings per hour and expressed as a percentage of the rate of the control (no LiCl added). Growth rates of controls of strain W3133-2 were 0.42 for lactic acid, 2.0 for tryptone, 1.0 for glucose, 0.49 for galactose, 0.91 for fructose, and 0.87 for glycerol. Control values for strain W3133-2S were similar to those for strain W3133-2. A, W3133-2 (wild type); B, W3133-2S (a mutant possessing an elevated activity of Li⁺ extrusion).](image)

![FIG. 1. Effect of Li⁺ on the growth of E. coli. Cells (W3133-2) were grown in a minimal salts medium supplemented with 10 mM melibiose (A) or 20 mM methyl-α-galactoside (B) in the absence (●) or presence (○) of 10 mM LiCl. OD$_{650}$. Optical density at 650 nm.](image)

<table>
<thead>
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<th>Cation</th>
<th>Pyruvate kinase activity (μmol of NADH utilized per min per mg of protein) in strain:</th>
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<tbody>
<tr>
<td></td>
<td>W3133-2</td>
</tr>
<tr>
<td>None</td>
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</tr>
<tr>
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<tr>
<td>Tl⁺</td>
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* Cations were added to a concentration of 50 mM as Cl⁻ salts except for Tl⁺ (SO₄²⁻ salt). SO₄²⁻ did not have a significant effect on pyruvate kinase. NT, Not tested.
Thus, it seems likely that Li⁺ produces some inhibitory effect on pyruvate kinase I by substituting for K⁺.

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