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Na^+ (Li^+)-Proline Cotransport in *Escherichia coli*

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Summary. Na^+ and Li^+ were found to stimulate the transport of L-proline by cells of *Escherichia coli* induced for proline utilization. The gene product of the put P gene is involved in the expression of this transport activity since the put P^+ strains CSH 4 and WG 148 show activity and the put P^- strain RM 2 fails to show this cation coupled transport. The addition of proline was found to stimulate the uptake of Li^+ and of Na^+. Attempts to demonstrate proline stimulated H^+ uptake were unsuccessful. It is concluded that the proline carrier (coded by the put P gene) is responsible for Na^+ (or Li^+)-proline cotransport.

Key Words proline transport · cation cotransport · harmaline

Introduction

The membrane transport of amino acids by bacteria is carried out by cation-substrate cotransport or by a mechanism involving a periplasmic binding protein plus a membrane protein. Many cation-cotransport systems use the proton as the cation, while others use the sodium ion. Early studies in *E. coli* suggested that this cell used exclusively H^+-amino acid cotransport systems (Harold, 1977). An exception to this generalization was discovered by Frank and Hopkins (1969) who suggested Na^+-glutamate cotransport in a mutant of *E. coli*.

For many years the proline carrier was regarded as one of the examples of H^+-cotransport because its transport was stimulated by a membrane potential (inside negative) in the absence of added Na^+ (Lombardi & Kaback, 1972; Hirata et al., 1973, 1974; Flagg & Wilson, 1978; Bentaboulet et al., 1979; Ten-Brink & Konings, 1980; Plate & Suit, 1981). On the basis of such experiments these authors believed that H^+-proline cotransport was the mechanism of uptake. Amanuma et al. (1977) measured the binding or proline to the carrier at different pH values and also concluded that the mechanism of transport was H^+-proline cotransport. Recent work from Anraku's laboratory (Mogi & Anraku, 1984*a–c*) suggests that 2 protons are transported for each proline molecule. In 1976 Kayama and Kawasaki reported that 10 mM Li^+ stimulated proline transport, although Na^+ addition had no effect. Furthermore, they failed to observe proton movement on addition of proline (Kayama-Gonda & Kawasaki, 1979). A further complexity was the finding that preincubation of membrane vesicles with Na^+ inhibited proline transport (Lombardi & Kaback, 1972; Hirata et al., 1974; Morikawa et al., 1974). Recently, Stewart and Booth (1983) have clarified many of the conflicting views by showing that the preincubation of cells with Na^+ leads to intracellular accumulation of this ion and inhibition of proline transport. On the other hand, they showed that the addition of Na^+ to the external medium simultaneously with radioactive proline led to the stimulation of uptake of this amino acid. Na^+ in the external medium increased the V_{max} of entry without affecting the K_m. These authors concluded that their data were consistent with Na^+-proline cotransport. Recently Tsuchiya et al. (1984) demonstrated proline induced Li^+ uptake in *E. coli*.

This communication confirms the observations of Stewart and Booth (1983) and of Tsuchiya et al. (1984). In addition it demonstrates directly the movement of Na^+ and Li^+ into the cell on the addition of proline. Experiments with CCCP demonstrate proline uptake in the presence of an inwardly directed electrochemical gradient for Na^+ in the absence of a protonmotive force. All attempts to demonstrate H^+ uptake on the addition of proline have failed.

Materials and Methods

Bacterial Strains and Growth Conditions

Strains of bacteria used in this study are listed in Table 1. CSH 4, the parental strain for two of the mutants, is F^- trp lacZ rpsL thi.

Table 1. Bacterial strains

Strain	Relevant genotype	Source
E. coli		
CSH 4	put A^+ put P^+ pro P^+ lac Z^-	Miller (1972)
WG 148	put A^- put P^+ pro P^- lac Z^-	Stalmach et al. (1983)
WG 148 (Z)[a]	put A^- put P^+ pro P^- lac Z^+	This paper
RM 2	put A^- put P^- pro P^+ lac Z^-	Menzel
X71-15	put A^+ put P^+ pro P^+	Kusch and Wilson (1973)

[a] A lactose positive revertant of WG 148.

Strain WG 148 was constructed from CSH 4 by Stalmach et al. (1983); RM 2 is a mutant derived from CSH 4 with a spontaneous deletion in put A and put P genes isolated by Rolf Menzel. The growth medium was Medium 63 (Cohen & Rickenberg, 1956) which contains 0.1 M potassium phosphate, pH 7.0, 15 mM $(NH_4)_2SO_4$ and 0.8 mM $MgSO_4$. The Na^+ content of Medium 63 was found to be 95 to 120 μM by atomic absorption spectroscopy (Perkin Elmer 5000). The carbon source was either 5 mg/ml L-proline or 1% Na succinate, plus 5 mg/ml L-proline; in one experiment 1% tryptone (Difco) plus 5 mg/ml L-proline were the carbon sources. Strains CSH 4, WG 148 and RM 2 required 50 μg/ml L-tryptophan. Thiamine (0.5 μg/ml) was added to all cultures. Cells were harvested in logarithmic growth phase, centrifuged and washed three times in Medium 63 or 250 mM TRIS-MES, pH 6.

Proline Uptake Measurement

Washed cells were suspended in buffer [Medium 63 or 250 mM Tris (hydroxymethyl) aminomethane-4-morpholineethyanesulfonic acid (TRIS-MES), pH 6], containing chloramphenicol (50 μg/ml) at a cell concentration of about 1 mg dry wt/ml. Cells were mixed with an equal volume of the same buffer containing [^{3}H]-proline to give a final concentration of 5 μM (0.5 μCi/ml) with or without added Na^+. Incubation was at 22°C. Samples (0.1 ml) were removed at various times, filtered on a 0.45 μm Millipore filter and washed with 5 ml of ice-cold buffer. The filter (plus cells) was place in a small plastic vial, 4 ml of liquid scintillation fluid was added and the sample counted. In some experiments the intracellular concentration was calculated, assuming 1 ml cell suspension of optical density 100 on a Klett-Summerson colorimeter (No. 42 filter) corresponds to 0.6 μl intracellular H_2O (0.22 mg dry wt).

Measurement of Cation Movement

Cells were grown in 1% tryptone, 5 mg/ml L-proline, 50 μg/ml L-tryptophan and 5 μg/ml thiamine. Cells were harvested in later logarithmic phase and washed three times with 100 mM Tris-3-[N-morpholino]propanesulfonate (MOPS) (pH 7.0). For Na^+ uptake experiments cells (2 mg dry wt/ml) were incubated in 10 ml of 100 mM tetramethylammonium-N-Tris(hydroxymethyl)-methylglycine (TRICINE) and brought to pH 8 with tetramethylammonium hydroxide (TMA-OH) plus 10 μM NaCl. Cells were incubated at 22°C in a plastic vessel fitted with a plastic cap with two holes through which calomel and Na^+ electrodes were passed (Tsuchiya & Wilson, 1978). Two additional very small holes were present in the cap, one for introducing N_2 gas and the other for introducing solutions. After 45-min incubation 10 μl of 100 mM L-proline was introduced and the pNa^+ followed on a chart recorder.

For Li^+ experiments cells (8 mg dry wt/ml) were placed in 3 ml of 100 mM TRIS-MOPS (pH 7) plus 100 μM LiCl. Incubation was carried out in a small plastic vial containing a Li^+-sensitive electrode (Tsuchiya et al., 1983). Nitrogen gas was introduced through a small hole in the cap and a second hole allowed exit of the N_2 and introduction of solutions. L-Proline (10 μl of 36 mM) was added and pLi^+ was recorded.

For H^+ uptake experiments cells (8 mg dry wt/ml) were placed in 3 ml of 150 mM choline chloride plus 20 mM KSCN. pH was monitored. L-Proline (10 μl of 30 mM) was added and pH followed.

Table 2. Effect of preincubation of Na^+ on proline uptake[a]

Experimental condition	Proline uptake in 20 sec (nmol/min/mg dry wt)
Control	1.62
Preincubation with 60 mM NaCl	0.36
Preincubation with 20 mM Na_3PO_4	0.36
60 mM NaCl in transport assay	3.84
20 mM Na_3PO_4 in transport assay	3.81

[a] Cells (WG 148) grown in Medium 63 with proline as carbon source were centrifuged and washed three times with 250 mM TRIS-MES buffer, pH 6. In the preincubation experiment 300 μl of cells (1.7 mg dry wt/ml) were incubated in the presence of 250 mM TRIS-MES buffer, pH 6, containing 60 mM NaCl or 20 mM Na_3PO_4 at 22°C for 5 min. Transport was then initiated by the addition of 300 μl of 250 mM TRIS-MES buffer, pH 6, containing 10 μM [^{3}H]-L-proline (1 μCi/ml) and NaCl or Na_3PO_4 at the same concentration as the preincubation buffer. In the second part of the experiment cells were incubated in the absence of added Na^+ salts for 5 min. Transport was initiated by the addition of an equal volume of buffer containing [^{3}H]-L-proline plus 120 mM NaCl or 40 mM Na_3PO_4. Samples (100 μl) were filtered, washed and counted. In the control experiment no Na^+ salts were added. To determine the concentration of Na^+ contaminating the incubation solutions to the control experiment, cells plus buffer (no proline) were filtered through 0.65 μm pore size Millipore filter and Na^+ concentration of the filtrate was found to be 10 μM.

Chemicals

L-Proline, chloroamphenicol, potassium cyanide, choline chloride, Tris(hydroxymethyl)aminomethane (TRIS), tetramethylammonium hydroxide (TMA-OH), 3-(N-morpholino) propanesulfonic acid (MOPS), N-Tris(hydroxymethyl)methylglycine (TRICINE), and harmaline hydrochloride were purchased from Sigma Chemical Company (St. Louis, Mo.); sodium chloride, lithium chloride and sodium succinate were obtained from Mallinckrodt (St. Louis, Mo.). Carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), was from Calbiochem-Behring (San Diego, Calif.). Tryptone was purchased from Difco (Detroit, Mich.). All phosphates were from Fisher Scientific (Boston, Mass.). [^{3}H]-L-Proline was purchased from Amersham (Arlington Heights, Ill.).

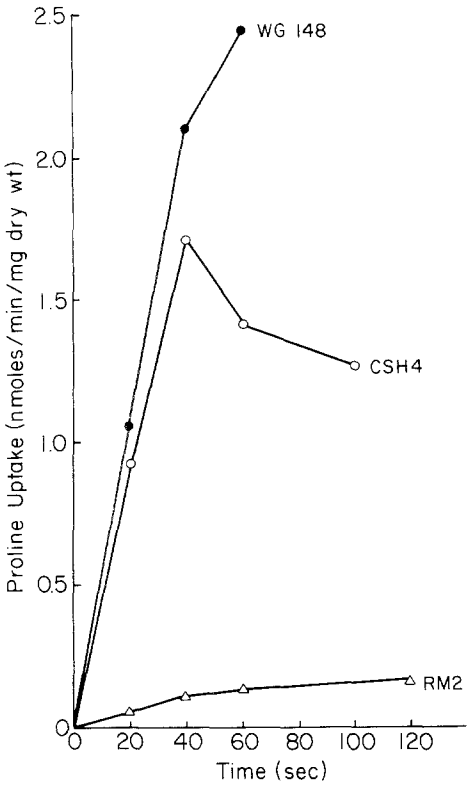

Fig. 1. Proline transport in *E. coli* strains with different genetic background. Cells of WG 148, CSH 4, and RM 2 were grown in Medium 63 with 5 mg/ml L-proline and 1% sodium succinate. Cells were centrifuged and washed three times with cold 250 mM TRIS-MES buffer (pH 6.0). Cells were resuspended with the same buffer containing 0.05 mg/ml chloroamphenicol. Transport was initiated by the addition of cells to an equal volume of 250 mM TRIS-MES buffer, pH 6.0 containing 10 mM Na_2SO_4 and 5 μM [^{3}H]-L-proline (0.5 μCi/ml). Symbols: WG 148, ●; CSH 4, ○; RM 2, △

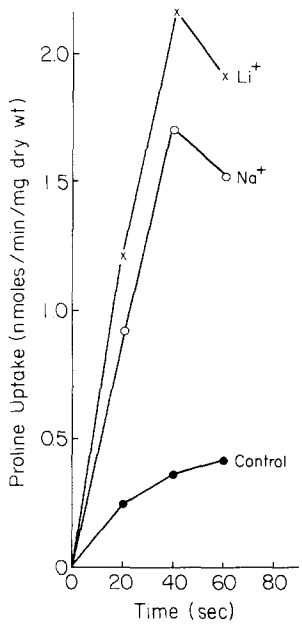

Fig. 2. Na^+ and Li^+ stimulation of the proline uptake in CSH 4. Cells were grown in Medium 63 with 5 mg/ml L-proline and 1% Na^+ succinate. Cells were harvested by centrifugation, washed three times with 250 mM TRIS-MES (pH 6.0), and resuspended in the same buffer containing 0.05 mg/ml chloroamphenicol. Cells were diluted 10-fold into 250 mM TRIS-MES (pH 6) buffer plus one of the three cations. 10 mM Na^+, Li^+ or choline$^+$ (anion was SO_4^{-2}) and 5 μM [^{3}H]-L-proline (0.5 μCi/ml). Symbols: 10 mM Li^+, ×; 10 mM Na^+, ○; and 10 mM choline$^+$, ●

Results

Effect of Na^+ on Proline Uptake

The first experiment was to test the effect of preincubating cells with Na^+ on the subsequent uptake of proline. Induced cells were washed with TRIS-MES buffer to remove traces of Na^+. Samples were then incubated for 5 min in the same buffer with or without Na^+ prior to the addition of proline. Uptake of [^{3}H]-proline was inhibited by preincubation with Na^+ (Table 2). In contrast, Na^+ added simultaneously with the [^{3}H]-proline caused a 2.4-fold stimulation of amino acid uptake. These results are in confirmation of the observations of Stewart and Booth (1983), and are consistent with the view that while Na^+ externally stimulates, Na^+ inside the cell inhibits.

The purpose of the next experiment was to determine which of the two known systems for proline transport was responsible for the Na^+-stimulated proline uptake. Figure 1 shows the proline transport activity (in the presence of 10 mM Na^+) in three different strains. Strain WG 148 possesses proline porter I (put P^+), lacks the enzyme proline dehydrogenase (put A^-) and lacks the proline porter II (pro P^-). Strain CSH 4, the wild-type parental strain, shows the same initial rate of proline uptake (30 and 45 sec points) as WG 148 but then shows a decline in radioactivity. The decline in radioactivity in the wild-type strain is believed by Morikawa et al. (1974) to be due to metabolism of proline and loss of the metabolic products from the cell. Strain RM 2, which lacks the put P gene, shows very low uptake.

Figure 2 shows the time course of proline uptake into strain CSH 4 in the presence or absence of 10 mM Na^+ or 10 mM Li^+. The 20-sec uptake of proline in the presence of Na^+ was 3.5-fold faster than the control; uptake in the presence of Li^+ was fourfold greater than the control.

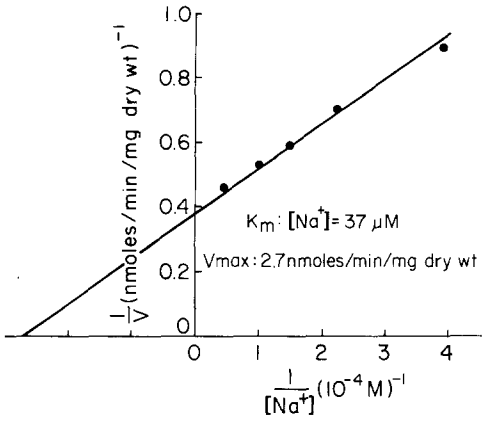

Fig. 3. Effect of different Na^+ concentrations on proline transport. Cells (X71-15) were grown in Medium 63 with 5 mg/ml L-proline as carbon source, harvested, washed and resuspended as described in Fig. 2. The Na^+ concentrations in different assays varied from 20 to 220 μM. The transport rate during the first 20 sec was taken as the initial rate. The data are expressed as a double reciprocal plot of 1/velocity and 1/Na^+ concentration

The effect of Na^+ concentration on [^{3}H]-proline uptake was next measured (Fig. 3). The K_m value for Na^+ stimulation was 37 μM Na^+; the V_{max} was 2.7 nmol/mg dry weight/min, a value similar to that observed by other workers (Stewart & Booth, 1983).

An attempt was next made to measure Na^+-stimulated proline accumulation in the absence of an electrochemical potential difference of protons. In the first half of the experiment cells incubated overnight in buffer were exposed to [^{3}H]-proline in the absence of 20 mM Na^+ with or without CCCP (Fig. 4*A*). The proton conductor, CCCP, strongly inhibited uptake. Under these conditions the ionophore would be expected to greatly reduce the membrane potential and thus decrease the electrochemical potential difference for both Na^+ and H^+. From this experiment alone, therefore, one could not determine which ion might be involved for cotransport.

In order to establish an inwardly directed Na^+ gradient in the absence of a protonmotive force, cells were energy depleted by exposing them to TRIS-MES buffer, pH 6, containing 5 μM CCCP plus 5 mM KCN overnight (16 hr) at 0°C. The purpose of this incubation was to equilibrate the internal and external pH (pH 6) as well as energy deplete the cell. Following this preincubation an aliquot of the concentrated cell suspension (with CCCP and KCN) was diluted 20-fold into pH 8 buffer containing 5 μM CCCP, 5 mM KCN, 100 mM NaCl and [^{3}H]-proline. Under these conditions protons would be expected to move out of the cell via the ionophore and produce an H^+ diffusion potential (inside negative). In the presence of this ionophore one would expect the H^+ to approach its electrochemical potential equilibrium. The driving force of the Na^+ ion would be inwardly directed due to both chemical gradient and the membrane potential. Cells treated in this manner showed uptake of proline reaching an internal concentration 18 times higher than the external medium at 16 min (Fig. 4*B*).

Another aliquot of energy-depleted cells was diluted into buffer at pH 6, 5 μM CCCP, 5 mM KCN, 100 mM NaCl and [^{3}H]-proline. In this experiment there was no pH gradient and thus no membrane potential. Once again the protonmotive force would be expected to approach zero. There was, however, a large chemical gradient favoring Na^+ entry. Under these conditions the entry of Na would be compensated electrically by the exit of H^+ on CCCP. Stimulation of proline was observed although less than the previous experiment (Fig. 4*B*).

The final aliquot was diluted into buffer at pH 6 containing 5 μM CCCP, 5 mM KCN and [^{3}H]-proline but with no added Na^+. Presumably there was a very low level of Na (perhaps about 10 μM), both inside and outside of the cell as a contaminant in the buffers. In this experiment there was no ion gradient for either Na^+ or H^+. A very low level of proline uptake was observed (Fig. 4*B*).

Effect of Harmaline

Harmaline is an organic base which has been shown to effect several Na^+-dependent processes in animal cells (Canessa et al., 1973; Sépulveda & Robinson, 1974). Proline transport was found to be strongly inhibited by harmaline. Table 3 shows the effect of this base on the proline transport in the presence of several cations. Na^+ and Li^+ at concentrations of 20 mM reduce the inhibitory effect of harmaline; Rb^+ and choline have no significant effect. The concentration of harmaline that gives 50% inhibition (I_{50}) with 90 μM Na was about 0.5 mM, while the I_{50} with Na^+ of 20 mM was greater than 5 mM harmaline. The data in Fig. 5 are given in the form of a Dixon plot and are consistent with competitive effects of Na^+ and harmaline.

Cation Movement

If the mechanism of proline uptake were Na^+-proline cotransport, it should be possible to demonstrate Na^+ uptake by cells during proline entry. Washed cells (of strains CSH 4, WG 148 and RM 2, were incubated in TRIS-MES buffer under anaerobic conditions. The sodium concentration was monitored continuously with an Na^+ electrode. A small

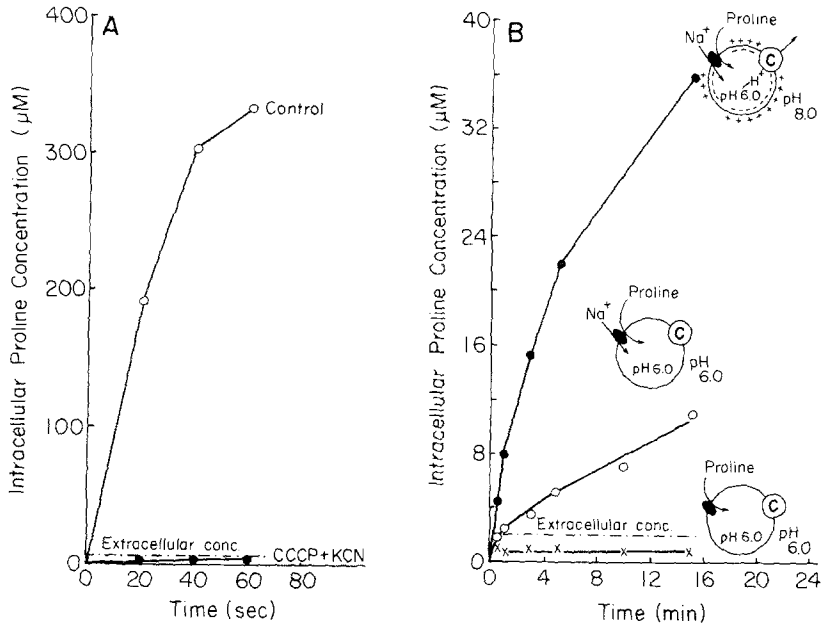

Fig. 4. Proline accumulation in the presence of Na^+ electrochemical gradient and the absence of protonmotive force. Cells (WG 148) were grown in Medium 63 and 5 mg/ml L-proline, centrifuged and washed three times in TRIS-MES buffer 250 mM, pH 6.0. Na^+ concentration in this buffer was examined by atomic absorption spectroscopy and found to be 20 μM. The cell suspension was divided into two parts. One was treated with 5 mM KCN and 5 μM CCCP; the other served as a control. Both were incubated on ice overnight. *A*. Following the overnight incubation control cells were added to 250 mM TRIS-MES, pH 6, 200 μM NaCl and 5 μM [^{3}H]-L-proline (0.5 μCi/ml with or without CCCP and KCN. ○, control; ●, 5 μM CCCP plus 5 mM KCN. *B*. CCCP-treated cells were centrifuged and resuspended in a concentrated suspension (optical density—12,000 Klett units) with the same buffer containing 5 μM CCCP and 5 mM KCN. Twelve microliters of this cell resuspension were diluted into 1.2 ml of each of the following solutions: 100 mM sodium phosphate buffer, pH 6.0; 100 mM sodium phosphate buffer, pH 8; and 250 mM TRIS-MES buffer at pH 6.0. Each of the above three solutions contained 2 μM L-proline (2 μCi/ml), 5 μM CCCP and 5 mM KCN. Two hundred microliters of this assay mixture were removed at various times, filtered and counted. Symbols: ●, 100 mM sodium phosphate, pH 8; ○, 100 mM sodium phosphate buffer, pH 6; and ×, 250 mM TRIS-MES buffer, pH 6.0

Table 3. Effect of cations on harmaline inhibition[a]

Salt added	Na^+ conc. in buffer (μM)	Proline uptake (nmol/mg dry wt/20 sec)	
		Control	Plus 2.2 mM harmaline
Choline Cl 20 mM	100	1.06	0.01
Rb Cl 20 mM	100	1.12	0.20
Na Cl 0.2 mM	100	1.00	0.30
Na Cl 20 mM	100	1.30	1.03
Li Cl 20 mM	100	1.03	0.71

[a] Cells X71-15 grown in Medium 63 and L-proline 5 mg/ml harvested, washed three times and resuspended in Medium 63 containing 0.05 mg/ml chloroamphenicol. Samples (10 μl) were taken at 20, 40 and 60 sec, filtered, washed and counted. Data at the 20-sec point are given in the table. Stock cells were added to an equal volume of solution containing radioactive proline, harmaline and NaCl. For example, 200 μl of stock cells (1.76 mg dry wt/ml) were mixed with 200 μl of Medium 63 containing 10 μM [^{3}H]-L-proline (1 μCi/ml), 4.4 mM harmaline and 40 mM NaCl. The final concentrations were 5 μM proline, 2.2 mM harmaline and 20 mM NaCl.

volume of anaerobic proline solution was added to give a final concentration of 100 μM. For strain CSH 4 there was an immediate fall in the concentration of Na^+ of the external medium, presumably as a result of the Na^+ entry into the cells in cotransport with proline (Fig. 6). A similar result was obtained with WG 148 (defective at put A and pro P). No such change was observed with strain RM 2 which possesses a deletion of genes put P and put A. This strain possesses proline porter II, which is known

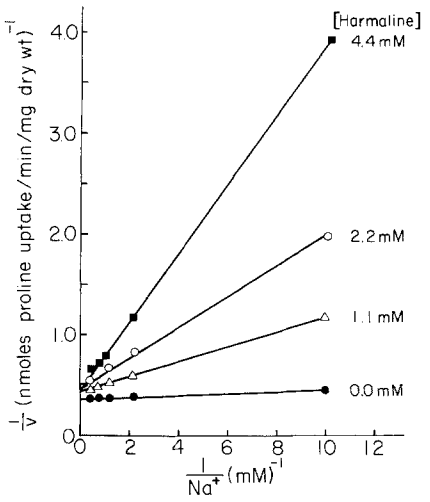

Fig. 5. Competitive interaction between harmaline and Na^+ on proline transport. Cells were grown in Medium 63 plus L-proline (5 mg/ml). Cells were centrifuged, washed three times in cold Medium 63, and resuspended in small volume of Medium 63 containing 0.05 mg/ml chloroamphenicol. Stock cells were added to an equal volume of [^{3}H]-proline solution containing harmaline and Na^+ as described in Table 3. Samples (100 μl) taken at 20, 40 and 60 sec were filtered, washed and counted. The initial rate of uptake was determined from this data. Similar experiments were carried out with four concentrations of harmaline (indicated on the graph) and five concentrations of Na^+ (100 μM, 500 μM, 1 mM, 2 mM, 10 mM)

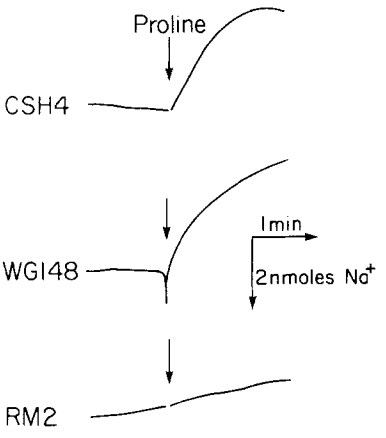

Fig. 6. Proline stimulation of Na^+ uptake. Cells of CSH 4, WG 148 and RM 2 were grown in Medium 63 with 5 mg/ml L-proline and 1% tryptone. The cells were harvested by centrifugation and washed three times with 100 mM TRIS-MOPS at pH 7.0. The assay mixture consisted of 10 ml of cells (2 mg dry wt/ml) with 100 mM Tricine-TMA-OH (pH 8.0), plus 10 μM NaCl. The sodium electrode was placed in the vessel and N_2 passed over the surface of the fluid. At the time indicated by the arrow 10 μl of L-proline (100 mM) were added. A rise in the curve indicates a fall in the external concentration of Na^+

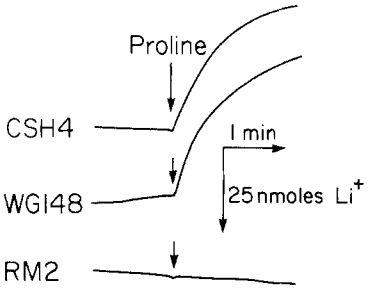

Fig. 7. Proline stimulation of Li^+ uptake. Cells of CSH 4, WG 148 and RM 2 were grown in Medium 63 with 5 mg/ml L-proline and 1% tryptone. The cells were harvested by centrifugation and washed three times with 100 mM TRIS-MOPS at pH 7. The assay mixture consisted of 3 ml of cells (8 mg dry wt/ml) with 100 mM TRIS-MOPS, pH 7.0, plus 100 μM lithium chloride. The lithium electrode was placed in the vessel and N_2 passed over the surface of the solution as described in Materials and Methods. After 40-min incubation 10 μl of 30 mM L-proline were added (at the arrow). A rise in the curve indicates a fall in the external concentration of Li^+

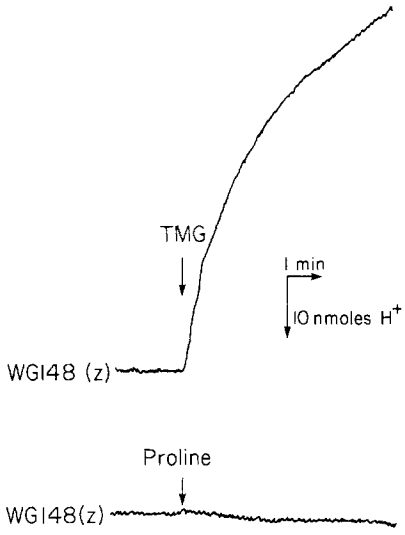

Fig. 8. pH changes following the addition of TMG and of proline. Cells of WG 148(Z), a lac^+ revertant of WG 148, were grown in Medium 63 plus 5 mg/ml L-proline and 1% Na^+ succinate with or without 1 mM IPTG (an inducer for the lactose carrier). Cells were harvested, washed three times with 120 mM choline chloride and resuspended in the same solution at a concentration of 20 mg dry wt/ml. In the upper curve 0.4 ml of cells (IPTG-induced) were mixed with 2.6 ml of 120 mM choline chloride containing 10 mM KSCN. The pH electrode was introduced into the vessel and N_2 was passed over the surface of the solution. After 1-hr incubation anaerobically at 22°C 30 μl of 1 M TMG were added. An upward deflection indicates a rise in pH. In the lower curve cells grown in proline and succinate (in the absence of IPTG) were incubated anaerobically in the same manner described above. After 1-hr incubation 10 μl of 30 mM L-proline were added

to show much lower activity than proline porter I after growth in proline-containing, tryptophan-sufficient medium (Wood 1981; Stalmach et al., 1983).

A similar experiment was carried out with Li^+ (in the absence of Na^+), the ion movement recorded with an Li^+ electrode. The addition of proline caused Li^+ uptake in CSH 4 and WG 148, but not RM 2 (Fig. 7).

Attempts were made to demonstrate proton uptake on the addition of proline. In one experiment (Fig. 8) a control was included to demonstrate the well-known H^+-galactoside cotransport. Cells induced for the lactose operon were incubated anaerobically and the lactose analog thiomethylgalactoside (TMG) was added. A marked alkalinization of the medium was observed as a result of H^+-TMG cotransport into the cell. The same bacteria grown in the absence of IPTG were tested in exactly the same manner except proline was added. No detectable proton uptake was observed.

Discussion

The failure to recognize Na^+-proline cotransport for many years was due to several factors. The presence of low levels of Na^+ contaminating many inorganic salts and the well-known leeching of Na^+ from glassware make it difficult to carry out experiments with Na^+ concentrations lower than approximately 50 to 100 μM when working with glass containers. In view of the low K_m for Na^+ (37 μM) the presence of 50 to 100 μM Na^+ may have involved Na^+-proline cotransport. The Li^+ stimulation of proline transport in the experiments of Kayama and Kawasaki (1976) provided a clue that cations other than H^+ might be involved. Stewart and Booth were the first to clearly demonstrate a Na^+ stimulation of proline uptake and recognize that Na^+ inside the cell inhibited the carrier for proline.

The experiments of this paper confirm the observations of these workers and demonstrate that Na^+ stimulates proline transport in the absence of a protonmotive force. In addition direct evidence of Na^+ and Li^+ uptake in the presence of proline is provided. All attempts to demonstrate proline stimulated proton uptake have failed.

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