Vol. 126, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1985 Pages 442-449

RESTORATION OF CELL VOLUME AND THE REVERSAL OF CARBOHYDRATE TRANSPORT AND GROWTH INHIBITION OF OSMOTICALLY UPSHOCKED Escherichia coli

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Received December 4, 1984

SUMMARY. Resumption of growth in osmotically upshocked Escherichia coli was effected only by an external stimulus (betaine treatment) in severe upshock, but was spontaneous in less severe upshock. In either case, growth resumption was preceded by a reversal of glucose transport inhibition, and that reversal was preceded by a recovery of cell volume. We hypothesize that deformation of the membrane by osmotic stress results in conversion of a membrane component of the transport system to a less functional conformation, which results in the inhibition of transport and the consequent inhibition of growth. Relief of the deformation would then allow recovery to a more functional conformation, reversal of transport inhibition, and then resumption of growth.

In the preceding report we showed that osmotic stress drastically inhibited active transport of carbohydrate by Escherichia coli, and that this transport inhibition was sufficient to account for the inhibition of growth caused by osmotic stress. In this report, an adaptive response to restore glucose transport, and thus growth, is shown to occur in osmotically upshocked cells. Drawing on our observations we present an initial working hypothesis on how membrane deformation, resulting from changes in cell volume, can account for the inhibitory action of osmotic upshock on glucose transport at the molecular level and on how that inhibition is reversed.

METHODS. E. coli CA8000 was grown as described in the preceeding report except that glucose was used as the carbon source in all experiments. Optical density was measured, protein and glucose were determined, rates of glucose utilization were calculated, and α-methylglucoside uptake assays were performed as described in the preceeding report. The glucose-glycerol diauxic lag time, the minimum time of exposure to glycerol in the absence of glucose before glycerol can be metabolized, was determined for E. coli CA8000 according to the procedure of Loomis and Magasanik (1). The cultures contained a growth-limiting amount of glucose (1.25 mM) plus either 0.05 or 0.8 M glycerol. With both cultures, growth stopped when glucose was exhausted, and

0006-291X/85 $1.50

growth owing to glycerol metabolism did not begin until 1.25 h later. E. coli CA8000 could not metabolize xylitol; no growth occured when 20 mM xylitol was supplied as the sole source of carbon and energy. E. coli CA8000 could not use betaine either as a source of carbon or as a source of nitrogen.

RESULTS. Betaine stimulated both glucose utilization (Fig. 1) and growth (Fig. 1, and references 2 and 3) in severely upshocked cells. Glucose utilization increased in the betaine-treated upshocked cells about a half hour before growth resumed (Fig. 1). This observation is evidence that the stimulation of glucose transport was not dependent on the increase in growth. Stronger evidence is that even in nongrowing upshocked cells, betaine increased the rate of α-methylglucoside uptake. The rate of uptake in nitrogen-starved stationary phase cells incubated for 3 hours in the presence of 0.8 M NaCl was 86 pmol/mg of protein/min; a rate of 474 was obtained when 2 mM betaine was also present during the incubation. Apparently, a reversal of the inhibition of glucose transport preceeded the ability to grow in cells exposed to severe osmotic stress.

During the severe osmotic stress caused by treatment with 0.8 M NaCl, the cells did not recover the capacity for either glucose transport or growth

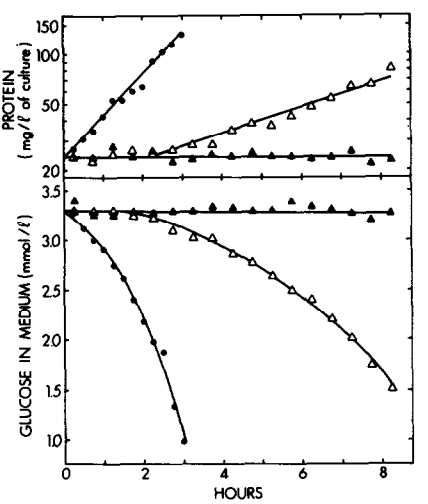

Fig. 1. Effect of osmotic upshock on E. coli CA8000: growth, assessed by the protein content of the culture, and glucose utilization. No addition (●), 0.8 M NaCl (▲), 0.8 M NaCl plus 2 mM betaine (△). The culture was grown and at zero time was treated with NaCl as described in Methods; betaine was added 10 minutes later. The time in which protein doubled and the rate of glucose utilization were 1.23 h and 12.4 mmol/g of protein/h in the control culture, and 3.6 h and 6.5 mmol/g of protein/h in the betaine-treated culture after growth resumed.

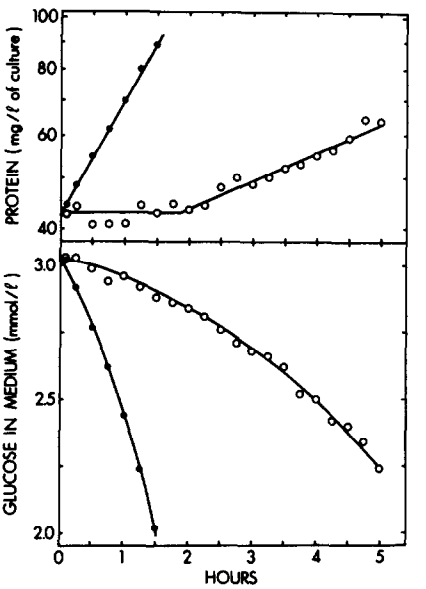

Fig. 2. Recovery of E. coli CA8000 during less harsh osmotic stress: growth, assessed by the protein content of the culture, and glucose utilization. No addition (●), 0.6 M NaCl (o). The culture was grown and treated as described in Methods. The time in which protein doubled and the rate of glucose utilization were 1.43 h and 10.7 mmol/g of protein/h in the control culture, and 5.7 h and 3.8 mmol/g of protein/h in the upshocked culture after growth resumed.

unless recovery was actuated by an external stimulus, betaine addition (Fig. 1). However, in the less severe conditions caused by 0.6 M NaCl, both growth and glucose utilization increased spontaneously (Fig. 2). During the first 2-hour period, growth was completely stopped. However, during this period the upshocked cells continued to use glucose (Fig. 2). In fact, the rate of glucose utilization appeared to be increasing during this first 2 hours, from about 1.6 to 3.3 mmol/g of protein/h. This increase in glucose transport in the nongrowing cells is more clearly demonstrated by the more sensitive measure of glucose transport, α-methylglucoside uptake. The rate of this uptake increased 2.6-fold during the first 2 hours after cells were upshocked with 0.6 M NaCl (Table 1). Thus, in the cells exposed to the less harsh osmotic stress of 0.6 M NaCl, a spontaneous reversal of the inhibition of glucose transport preceeded the ability to grow.

In the absence of growth, changes in the optical density of a culture reflect changes in cell volume (4-7). These two changes are related in a reciprocal manner, i.e., an increase in optical density reflects a decrease

Table 1. Spontaneous reversal of inhibition of α-methylglucoside uptake in 0.6 M NaCl

Addition	Incubation time (h)	Rate of uptake (pmol/mg of protein/min)
None	0.25	945
	2.0	1030
0.6 M NaCl	0.25	123
	0.5	167
	1.0	246
	1.5	283
	2.0	320

Uptake assays were performed with E. coli cells as described in Methods after room temperature (24°C) incubation for the times listed below.

in cell volume and a decrease in optical density reflects an increase in cell volume. Thus, from the optical density changes after upshock with 0.8 M NaCl (Fig. 3) one can see that cell volume decreased and then slowly increased, but never approached the original cell volume. These upshocked cells that did not regain any significant fraction of the original cell volume also did not regain the capacity for glucose transport or growth (Fig. 1). However, cells upshocked with 0.8 M NaCl in the presence of 2 mM betaine showed an initial volume decrease, but then rapidly increased in volume until the original cell volume was almost regained (Fig. 3). Betaine apparently entered the cells and equalized the osmolarity on both sides of the membrane allowing volume restoration. (Upshocked E. coli concentrate exogenous betaine intracellularly (2).) During the next 1.5 hours after this restoration of volume, glucose transport and then growth resumed (Fig. 1). Apparently, glucose transport and growth cannot resume in upshocked cells unless a significant fraction of the cell volume is first restored.

With cells upshocked with 0.6 M NaCl, cell volume was restored spontaneously (data not shown) and then glucose transport and growth were restored spontaneously (Fig. 2). The spontaneous recovery of volume with a lesser degree of upshock may be effected by an increased uptake of potassium. This ion is known to accumulate in osmotically upshocked cells (8,9), and this accumulation, by helping to balance internal and external osmolarity, is known

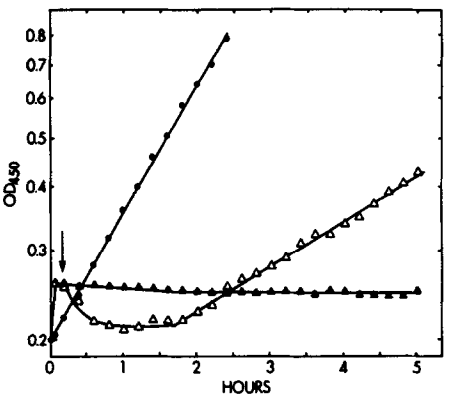

Fig. 3. Effect of 0.8 M NaCl and betaine on the optical density of E. coli CA8000: no addition (●), 0.8 M NaCl (▲), 0.8 M NaCl plus 2 mM betaine (△). Betaine was added at 10 minutes (arrow). The culture was grown and upshocked as described in the legend to Fig. 1. In the absence of growth, changes in the optical density of a culture are reciprocally related to changes in cell volume (4-7). The culture treated with 0.8 M NaCl did not grow throughout the experiment; the betaine-treated culture did not grow for approximately 2 hours (e.g., see Fig. 1). The time in which the optical density doubled was 1.20 h during the growth of the control culture, and 3.4 h in the betaine-treated culture after growth resumed.

to lessen the change in membrane shape that occurs in hypertonic solutions of impermeant solutes (10).

We tested the effect of potassium on restoration of cell volume and of glucose transport. In the presence of potassium, cell volume was almost totally restored by 15 minutes after upshock with 0.2 M xylitol, but in the absence of potassium cell volume was not fully restored by 15 minutes after upshock (Table 2). In addition, the presence of 1 mM KCl in the upshock medium prevented nearly half of the inhibition of α-methylglucoside uptake activity that occurred 15 minutes after the addition of 0.2 M xylitol to the assay medium (Table 2). Thus, restoration of cell volume was again accompanied by a restoration of glucose transport.

Another observation strongly supports the concept that the inhibition of transport and growth by hypertonicity is related to the shrinkage of the cell volume. A hypertonic solution of glycerol, a freely permeant sugar alcohol that does not affect cell volume (4), had no effect on α-methylglucoside uptake and only a minimal effect on growth. The rate of α-methylglucoside uptake in the presence of 0.8 M glycerol was 993 pmol/mg of protein/min compared to 1000 in the absence of glycerol; the specific growth rate was de-

Table 2. Protective effect of potassium ion on α-methylglucoside uptake by upshocked cells

Addition	OD_{450}[a]		α-methylglucoside uptake	
	1 min	15 min	Rate (pmol/mg of protein/min)	Activity remaining (%)
None	0.200	0.195	659[b]	—
1 mM KCl	0.196	0.198	680	103
0.2 M xylitol	0.223	0.209	191	29
0.2 M xylitol plus 1 mM KCl[c]	0.214	0.197	388	59
0.8 M glycerol	0.187	0.186	640	97
0.8 M NaCl	0.242	0.234	311	47

In order to evaluate the effect of potassium, the cells were suspended and uptake assays were performed in a potassium-free medium (100 mM Tris-HCl, pH 7.0) instead of the potassium-containing essential salt solution used for all of the other uptake assays. All other procedures were performed exactly as described in Methods. Rates of uptake were measured after a 15-minute room-temperature incubation in the Tris buffer containing the various agents listed below.

[a]Optical density and cell volume are not related in a simple reciprocal manner, but rather are related in a reciprocal exponential manner (5). Thus, small relative differences in optical density reflect larger relative differences in cell volume.

[b]The rate of uptake was lower in the Tris buffer used here than in the essential salt solution (see Tables 1 and 3) for reasons that are not known at present. Despite this lower rate, the Tris buffer does appear to be similar to the essential salt solution, because 0.8 M glycerol did not (and 0.8 M NaCl did) inhibit transport in either system (see text and Fig. 1).

[c]Total restoration of cell volume was not accompanied by complete restoration of transport. Apparently in the Tris buffer some permanent modification of the membrane remains even after cell volume is restored.

creased only about 10%, from 0.83 to 0.74 doublings/h, by the addition of 0.8 M glycerol.[1] In contrast, exposure to an 0.8 M solution of the impermeant solute NaCl, which caused plasmolysis and loss of cell volume (Fig. 3), almost totally inhibited glucose transport and totally inhibited growth (Fig. 1).

Osmotic upshock made α-methylglucoside uptake susceptible to inhibition by N-ethylmaleimide (Table 3). Uptake of α-methylglucoside by untreated

[1]The small effect of hypertonic glycerol on growth and the lack of effect on α-methylglucoside uptake cannot be ascribed to metabolism of glycerol by the glucose-grown cells used in these experiments. Such cells cannot metabolize glycerol until after 1.25 h of exposure to glycerol in the absence of glucose (see Methods). Uptake of α-methylglucoside was measured after only 15 minutes of exposure to 0.8 M glycerol, and glucose was present in the culture that was treated with 0.8 M glycerol to test its effect on growth.

Table 3. Sensitization of α-methylglucoside uptake to N-ethylmaleimide (NEM) by osmotic upshock, and reversal of both NEM inhibition and cell volume changes by betaine

Addition	OD_{450}		α-methylglucoside uptake	
	1 min	15 min	Rate (pmol/mg of protein/min)	Decrease (%)
None	0.200	0.195	988	—
2 mM betaine	0.198	0.199	1040	0
0.2 mM NEM	0.200	0.198	1010	0
2 mM betaine + 0.2 mM NEM	0.199	0.196	1080	0
0.2 M NaCl	0.247	0.235	786	20
0.2 M NaCl + 2 mM betaine[a]	0.240	0.221	962	3
0.2 M NaCl + 0.2 mM NEM	0.245	0.234	542	45
0.2 M NaCl + 2 mM betaine + 0.2 mM NEM	0.238	0.221	751	24

Uptake assays were performed at room temperature with E. coli cells suspended in the essential salt solution (165 mOsmol/l) which contained the particular additions listed below. Rates of uptake were measured 15 minutes after the additions.

[a]The fact that transport activity was nearly fully restored by betaine, but cell volume appeared to be only partially restored, suggests that in the essential salt solution some degree of membrane deformation can take place without greatly affecting transport activity.

control cells in the essential salt solution showed little or no inhibition by 0.2 mM N-ethylmaleimide, but this same concentration inhibited uptake more than 50% in cells upshocked by adding 0.2 M NaCl to the essential salt solution (Table 3). The presence of an osmoprotectant, betaine, in the medium lessened the degree of inhibition by N-ethylmaleimide of α-methylglucoside uptake in the upshocked cells (Table 3). Thus, shrinkage of the cells appears to play a role in the inhibition of transport by N-ethylmaleimide.

DISCUSSION. Our observations suggest that the inhibition of glucose transport during osmotic upshock occurs because the change in membrane shape (which results from the change in cell volume) impairs a membrane component of the glucose transport (phosphotransferase) system. A shift in the conformational state of enzyme II, a membrane component of the system (11), alters both the rate of transport and the sensitivity of enzyme II to inhibition by N-ethylmaleimide (12-15). This sulfhydryl poison, when it is on the outside

of the cell membrane, can react with enzyme II only when it is in its less functional conformation (see Fig. 2 of reference 15). In osmotically upshocked cells, glucose transport was sensitized to inhibition by exogenous N-ethylmaleimide (Table 3). Thus, a reasonable initial working hypothesis is that hypertonic stress, which is known to cause plasmolysis and membrane deformation, causes enzyme II to be converted to its less functional conformation, which results in the observed inhibition of glucose transport and the consequent inhibition of growth.

Addition of betaine, a treatment that allowed a significant restoration of cell volume (Fig. 3 and Table 3), reversed the inhibition of transport in upshocked cells (Table 3). In addition, betaine prevented some of the inhibition of transport in upshocked cells by N-ethylmaleimide (Table 3). Thus, reversal of membrane deformation apparently allows enzyme II to be converted back to its more active conformation, resulting in a reversal of glucose transport inhibition and subsequently a resumption of growth.

ACKNOWLEDGEMENT. This work was supported by National Institutes of Heatlh Grants AM07153 and AM18670.

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