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AqpZ-Mediated Water Permeability in *Escherichia coli* Measured by Stopped-Flow Spectroscopy

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We report that the water permeability of wild-type *Escherichia coli* during exponential growth is comparable to that of an *aqpZ* disruption mutant. In contrast, an increase in permeability is observed for the wild type at the onset of the stationary stage with no significant corresponding change in the permeability of the mutant.

Membrane channels are thought to be required for the osmoregulation of plant, animal, and bacterial cells (8). Aquaporin Z (AqpZ), which was identified a decade ago in wild-type *Escherichia coli* on the basis of sequence homology to other aquaporins, was the first water channel to be recognized in prokaryotes (5). In contrast to earlier reports that expression of *E. coli aqpZ* peaks during mid-exponential phase (6), a recent study by Soupene et al. challenged the hypothesis that expression of the gene is an adaptive response by rapidly growing cells and instead found that high levels of transcription of *aqpZ* occurred during transition into the stationary growth phase. In enriched medium, increased transcription was under the control of the RpoS sigma factor (9). Although it has been previously demonstrated that purified *E. coli* AqpZ increases the permeability of water in liposomes (2, 3), there is a notable absence of direct evidence that AqpZ mediates water permeability in vivo. We report here the water permeability of wild-type *E. coli* and an *aqpZ* disruption mutant as a function of the stages of cell growth.

Relationship between growth rate and cell permeability of the wild type. Starter cultures of *E. coli* MG1655 (CGSC 7740) for our study were grown to full yield at 37°C in Luria-Bertani (LB) medium, and after 15 h they were diluted 1:100 into the same medium to initiate the experiments. At 1-h intervals, 30 ml of the culture was centrifuged for 5 min at 10,000 × *g* at 5°C, the cell pellet was washed twice with 10 ml 100 mM (pH 7.4) phosphate-buffered solution (PBS), and the final cell pellet was resuspended in 30 ml PBS. The entire manipulation was complete, and permeability measurements were made within 10 min of sampling the culture. We employed a HI-TECH SF61 DX2 and stopped-flow methods to determine the permeabilities (1, 7). *E. coli* cells were subjected to an outwardly oriented osmotic gradient (see below), and the change in light scattering at λ_{em} = 600 nm was recorded as a function of time. Two features were observed in the transient spectra that followed mixing: a rapid exponential increase in scattering and a subsequent slower exponential decrease in scattering (data not shown). These events are attributed to egress of water from the cytoplasm and a subsequent slower movement of the osmolyte into the cytoplasm, as indicated by the direction of the change in scattering, the similarities of the observed rate of the first event to previous measurements of the egress of water from *E. coli* (1), and the insensitivity of the rate of the first event (and a sensitivity of the second event) to the chemical nature of the osmolyte (see below). The magnitude of change in scattering was proportional to the concentration of the cells, which was adjusted to permit the transmission of 60 to 80% of the light. Proline was selected as the osmolyte for our initial studies because it is considered to be a "compatible solute" (it can be accumulated to very high levels without disturbing cellular physiology), and as such it is frequently used to balance environmental osmolality (4). The measurements that are summarized in Fig. 1 and Table 1 are for the 1:1 mixing of cells suspended in PBS and 1 M L-proline in PBS (to give a final concentration of 500 mM L-proline in 100 mM PBS). To further improve the signal-to-noise ratio by reducing the basal water permeability, our initial measurements were carried out at 5°C. The emission/time traces that were used to determine the rate constants were the average of five mixing cycles. The standard deviations that are reported in Table 1 were obtained through statistical comparison of five independent averaged spectra. The change in scattering due to egress of water and influx of osmolyte were fitted to single exponential functions to give the pseudo-first-order rate constants k_w and k_p, respectively. The osmotic permeabilities (P_f) were computed using the following equation: $P_f = [V_0/(S \times V_W \times \Delta_{osM})] \times d[V/V_0]/dt$, where the molar volume of water (V_W) is 18 cm^3/mol, the change in osmolality (Δ_{osM}) is 5×10^{-4} mol/cm^3, and $d[V/V_o]/dt$ is $k_{w/p}$. The initial volume (V_o) and surface area (S) of the cells were computed assuming a length of 5 μm and a diameter of 1 μm, which are average values for K-12 strains of *E. coli* grown in LB during exponential phase and at the beginning of stationary phase (10). Figure 1A illustrates the relationship between P_f for water at 5°C and the growth of *E. coli* MG1655 (wild type) in LB with aeration at 37°C. One observes an initial decrease in permeability of the cells during the transition from the lag phase to exponential growth, relatively little change in permeability during exponential growth itself, and then a rapid increase in permeability that coincides with the onset of the stationary phase. The initial decrease in permeability that is observed in Fig. 1A was eliminated in an experiment that was identical in every respect, except the experiment was initiated by subculturing a starter culture that was in the mid-exponen-

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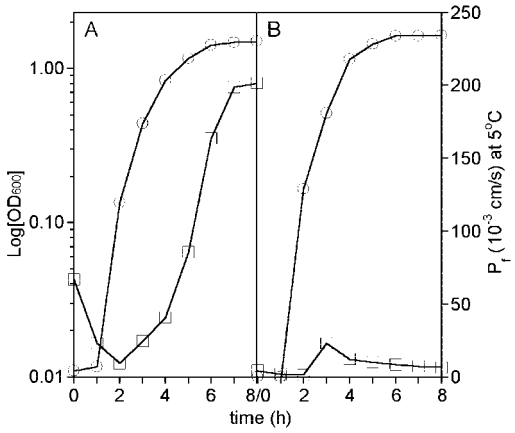

FIG. 1. Growth (open circles) of (A) MG1655 (wild type) and (B) NCM3306 (Δ*aqpZ*::Cam$^+$) in LB with aeration at 37°C (optical density at 600 nm [OD_{600}]) and osmotic permeability (open squares) for the efflux of water from cells suspended in PBS (100 mM Na_xPO_4, pH 7.4) upon 1:1 mixing with 1 M L-proline (in the same PBS) at 5°C as a function of time. The cultures were started by 1:100 dilution of a 15-h culture with fresh LB medium.

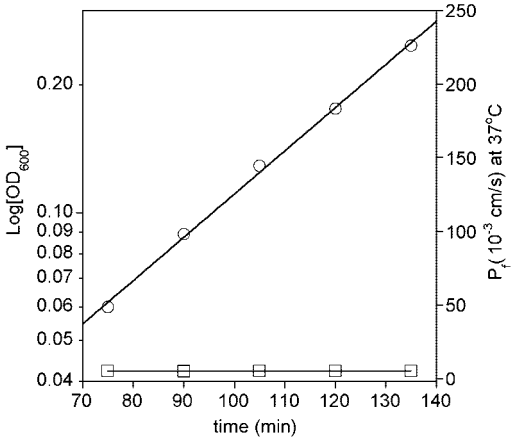

FIG. 2. Growth (open circles) of MG1655 (wild type) in LB with aeration at 37°C (optical density at 600 nm [OD_{600}]) during the exponential phase of growth and osmotic permeability (open squares) for the efflux of water from cells suspended in PBS (100 mM Na_xPO_4, pH 7.4) upon 1:1 mixing with 1 M L-proline (in the same PBS) at 37°C as a function of time. The line was generated by least-squares fit of the growth data (which yields a generation time [g] of 30 min).

tial growth phase rather than one that was in late stationary phase. The observed relationships between the rate of growth and permeability in Fig. 1A, most notably the initial decrease in permeability and the lag that occurs between the observed increase in permeability and the onset of exponential growth, is consistent with the observation that transcription of an *aqpZ-lac* fusion on the chromosome of *E. coli* increases as the cells enter the stationary stage of growth (9).

TABLE 1. Rate constants for the egress of water (k_w) and the influx of L-proline (k_p) for MG1655 (wild type) and NCM3306 (Δ*aqpZ*::Cam$^+$) for Δ_{osM} = 500 mosM at 5°C as a function of growth[a]

Strain and time (h)	k_w (s^{-1})	k_p (s^{-1})
MG1655		
0	27.0 (7)	0.97 (5)
1	9.2 (6)	0.57 (4)
2	3.70 (7)	—[b]
3	9.7 (4)	0.51 (5)
4	16.2 (2)	0.62 (3)
5	34 (1)	0.94 (4)
6	65 (1)	1.07 (9)
7	79 (1)	0.96 (4)
8	80 (2)	1.01 (6)
NCM3306		
0	1.75 (1)	—[b]
1	0.82 (3)	—[b]
2	0.62 (1)	—[b]
3	9.2 (6)	0.34 (4)
4	4.8 (2)	0.67 (1)
5	4.0 (1)	0.8 (1)
6	3.4 (2)	0.83 (2)
7	2.95 (4)	—[b]
8	2.5 (2)	—[b]

[a] Estimated error of the least significant digit is in parentheses.
[b] These rate constants could not be accurately measured due to overlap and/or weak signals.

Effect of AqpZ on cell permeability. AqpZ is not the only water-permeable membrane protein in *E. coli*. GlpF facilitates the permeation of both glycerol and water, although in vitro experiments suggest GlpF is significantly less effective at the transport of water than AqpZ (2). To rule out other physiological and morphological changes that might affect permeability, the experiment was repeated using NCM3306 (Δ*aqpZ*::Cam$^+$) (9), a derivative of MG1655 in which the *aqpZ* gene is disrupted. The experiment was carried out using the same protocol as that for the wild type. In contrast to the marked effect the stage of growth has on the rate of water efflux for the wild-type (Fig. 1A), P_f remains essentially constant throughout the stages of growth for the *aqpZ* knockout (Fig. 1B).

Effect of temperature on cell permeability. To confirm that permeability was constant during exponential growth and to ensure that the observed trends in permeability were not artifacts of the low temperature that was employed in our initial experiments (e.g., vis-à-vis phase transition of the lipids), in a subsequent experiment we collected more samples during exponential growth (at 15-min intervals instead of 1-h intervals) and the permeability measurements were carried out at 37°C. The more closely spaced sampling required a modification of our sampling protocols such that cell pellets were stored at 5°C until the permeability measurements could be made, typically within 45 min of sampling. The overall relationship between growth rates and permeability was similar at 37°C, although the basal and maximal permeabilities were somewhat higher, presumably due to greater passive diffusion of water. Figure 2 clearly demonstrates that basal permeability is maintained during the exponential growth of MG1655. A similar basal permeability was observed for NCM3306 at 37°C (data not shown).

Effect of osmolyte on cell permeability. The rate of water egress was dependent on the applied osmotic gradient but not the chemical nature of the osmolyte itself. Thus, proline, betaine, and trehalose, all considered to be "compatible osmo-

TABLE 2. Basal and maximal osmotic permeabilities of MG1655 (wild type) and NCM3306 (Δ*aqpZ*::Cam$^+$) for Δ_{osM} = 500 mosM at 37°C

Osmolyte	MG1655 P_f (10^3; in cm/s)	NCM3306 P_f (10^3; in cm/s)
Proline	14.0 (1)/93.0 (9)	15 (4)[a]
Betaine	16.3 (2)/88.4 (6)	
Trehalose	15.4 (1)/89.4 (7)	

[a] The average (n = 25) for measurements that spanned the lag growth phase, through the exponential growth phase, and into the stationary phase.

lytes" (4), yielded comparable basal and maximal permeabilities (Table 2).

Conclusions. The increased permeability for the wild type is apparently largely due to the incorporation of AqpZ into the cytoplasmic membrane. The permeability data we report here support the conclusion of Soupene et al. that expression of *aqpZ* increases as cell growth slows and the cells enters stationary phase (9). Our results also indicate that AqpZ mediates water permeability in vivo. However, these observations do not address the issue of whether AqpZ traffics exclusively in water or whether it might also mediate the permeability of other small molecules, for example, the fermentation end product ethanol, as suggested by Soupene et al. (9).

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