The Osmotic Activation of Transporter ProP Is Tuned by Both Its C-terminal Coiled-coil and Osmotically Induced Changes in Phospholipid Composition

From the ‡Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada and the §Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School, Houston, Texas 77030

Transporter ProP of Escherichia coli (ProPEc) senses extracellular osmolarity and mediates osmoprotectant uptake when it is rising or high. A replica of the ProPEc C terminus (Asp468–Arg497) forms an intermolecular α-helical coiled-coil. This structure is implicated in the osmoregulation of intact ProPEc, in vivo. Like that from Corynebacterium glutamicum (ProPCg), the ProP orthologue from Agrobacterium tumefaciens (ProPAT) sensed and responded to extracellular osmolarity after expression in E. coli. The osmotic activation profiles of all three orthologues depended on the osmolality of the bacterial growth medium, the osmolarity required for activation rising as the growth osmolality approached 0.7 mol/kg. Thus, each could undergo osmotic adaptation. The proportion of cardiolipin in a polar lipid extract from E. coli increased with extracellular osmolarity so that the osmolality activating ProPEc was a direct function of membrane cardiolipin content. Group A ProP orthologues (ProPEc, ProPAT) share the C-terminal coiled-coil domain and were activated at low osmolalities. Like variant ProPEc-R488I, in which the C-terminal coiled-coil is disrupted, ProPEc derivatives that lack the coiled-coil and Group B orthologue ProPCg required a higher osmolality to activate. The amplitude of ProPEc activation was reduced 10-fold in its deletion derivatives. The coiled-coil structure is not essential for osmotic activation of ProP per se. However, it tunes Group A orthologues to osmoregulate over a low osmolality range. Coiled-coil lesions may impair both coiled-coil formation and interaction of ProPEc with amplifier protein ProQ. Cardiolipin may contribute to ProP adaptation by altering bulk membrane properties or by acting as a ProP ligand.

Bacteria respond to changes in medium osmolality by modulating cytoplasmic composition (1–3). Osmoregulatory transporters and biosynthetic enzymes mediate the accumulation of K⁺, glutamate, and selected organic solutes as extracellular osmolarity increases. Mechanosensitive channels release solutes as osmolality decreases. Three osmoprotectant transporters were shown to act as both osmosensors and osmoregulators after purification and reconstitution in proteoliposomes: H⁺ symporter ProP of Escherichia coli (4), Na⁺ symporter BetP of Corynebacterium glutamicum (5), and ATP-binding cassette transporter OpuA of Lactococcus lactis (6). Each was activated as electrolytes were concentrated in the lumen of proteoliposomes, with or without osmotically induced proteoliposome shrinkage (7). In addition, the osmotic upshift required to activate BetP (5) or OpuA (8) increased with the mole fraction of the anionic phospholipid phosphatidylglycerol (PG) in the proteoliposome membrane. It was thus proposed that osmosensing occurs when osmotically induced changes in cytoplasmic ionic strength or K⁺ concentration alter transporter-lipid interactions (7, 9).

Transporter ProE of E. coli (ProPEc) mediates the uptake of zwitterionic osmoprotectants such as proline and glycine betaine (N-trimethylglycine) when osmolality is rising or consistently high (10). ProPEc is a 500-amino acid integral membrane protein and a member of the major facilitator superfamily (11). Our homology model of ProPEc (12) is based on the crystal structure of 12-transmembrane helix transporter GlpT (13), which shares a common fold with major facilitator superfamily members OxlT and LacY (14). Protein ProQ of E. coli amplifies ProPEc activity by acting post-translationally (15, 16). ProQ is a basic, cytoplasmic protein that may act directly or indirectly on ProPEc (17).

The central cytoplasmic loop (C3) and C terminus of ProPEc are longer than those of its paralogues (7, 12) (Fig. 1A). The latter terminates in six or seven of the heptad repeats that characterize α-helical coiled-coil-forming proteins (11). Studies of synthetic peptides corresponding to residues 456–500 (18) or 468–497 (19, 20) of ProPEc showed that residues 468–497 (encompassing four heptads) form an antiparallel, homodimeric α-helical coiled-coil of low stability (Fig. 1, B and C). The low stability of this structure was expected, because basic residues are present at core heptad "a" positions His495 and Arg488 (11) (Fig. 1C). Unexpectedly, replacement R488I disrupted coiled-coil formation by this peptide replica, providing the first evidence that the orientation of the coiled-coil might be antiparallel (18).

The antiparallel orientation was substantiated by the NMR solution structure, which appears to be stabilized by interactions of Arg488 with Asp475 and Asp478 on the opposing monomer strand (Fig. 1B) (20). This antiparallel structure was also detected in intact ProPEc, in vivo, by chemical cross-linking of introduced Cys residues (21). A higher osmolality was required to activate the R488I variant than wild type ProPEc, and the R488I variant was activated only transiently, whereas activation of wild type ProPEc was sustained indefinitely (18). These results suggested that the C-terminal coiled-coil of ProPEc plays a role in its osmotic activation.

Amino acid sequence comparisons reveal two groups of bacterial

‡ The abbreviations used are: PG, phosphatidylglycerol; CL, cardiolipin; PE, phosphatidylethanolamine; PC, phosphatidylcholine; MOPS, 4-morpholinopropanesulfonic acid; Tricine, H[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.


**Experimental Procedures**

**Culture Media**—E. coli strains were cultivated at 37 °C, whereas the Agrobacterium tumefaciens strain was cultivated at 30 °C, in LB medium (23) or in NaCl-free MOPS medium, a variant of the MOPS medium described by Neidhardt et al. (24), from which all NaCl was omitted. MOPS medium was supplemented with \( \text{NH}_4\text{Cl} \) (9.5 mM) as a nitrogen source and glycerol (0.4% v/v) as a carbon source. L-Tryptophan (245 \( \mu \text{M} \)) and thiamine hydrochloride (1 \( \mu \text{g/\muL} \)) were added to meet auxotrophic requirements, and NaCl or sucrose was added to adjust the osmolality as indicated. Ampicillin (100 \( \mu \text{g/mL} \)) was included to maintain plasmids, and arabinose was added as specified to adjust ProP expression.

**Bacteria, Plasmids, and Molecular Biological Manipulations**—Basic molecular biological techniques were as described by Sambrook and Russell (25). Chromosomal DNA was isolated as described by Baylor et al. (26). The PCR was carried out as described by Brown and Wood (27). Site-directed mutagenesis was performed using the QuikChange Mutagenesis Kit (Stratagene, La Jolla, CA) as described by Culham et al. (28). Oligonucleotides were purchased from Genescript (Kingston, Canada). Each recombinant plasmid was recovered from a ligation mixture by transformation of E. coli DH5a (29), and the entire sequence of the encoded proP variant was confirmed (GenAlEx, Guelph, Canada) before the plasmid was expressed in E. coli WG350.

Genes encoding ProP and its variants were expressed from the AraC-controlled P\(_{\text{BAD}}\) promoter in plasmid-bearing derivatives of E. coli WG350 (\( \text{F}^{-} \) trp lacZ rpsL thi ΔputPA101 Δ(proU)600 Δ(proPMetAB)212) (11). Each strain contained pBAD24 (30) or a pBAD24 derivative constructed as outlined above and by Tsatskis (31) or Khamati (32). These included pDC79 (encoding ProP Ec), pYT1 (encoding His\(_6\)-ProPCg), pYT12 (encoding ProPCg), and pYT13 (encoding ProPA).

**Isolation of Genes proPCg and proPA**—A BgIII site overlapping the proP Ec stop codon in pDC79 was introduced by site-directed mutagenesis, the resulting plasmid (pYT6) was cleaved with EcoRI and BgIII to...
excise proPEc, and the vector fragment was purified. The gene encoding ProPCg was PCR-amplified using plasmid pHPS (22) as template, and the gene encoding the putative Group A ProP orthologue from A. tumefaciens (ProPAI) was PCR-amplified using the linear chromosome of A. tumefaciens CS8 (ATCC number 33970) (American Type Culture Collection (Manassas, VA)) as template. During amplification, an EcoRI site was introduced 5’ to each open reading frame, and a BglII restriction site was introduced overlapping the stop codon. Each PCR product was digested with EcoRI and BglII, purified, mixed with the vector fragment of pYT6, and ligated with T4 DNA ligase to create plasmids pYT12 (encoding ProPCg) and pYT13 (encoding ProPAI).

**C-terminal Truncation of ProPEc**—The proPEc gene encoded by pYT1 was PCR-amplified so that the introduced EcoRI site 5’ to the open reading frame was included, and a stop codon, with an overlapping BglII site, was introduced after the codon for Ala582 or Thr489. The resulting PCR products and plasmids pYT6 were cleaved with EcoRI and BglII. The desired DNA fragments were purified, mixed, and ligated, creating plasmids pMD2 (encoding His6-ProP Ec (Glu440–Glu500) on a Western blot).

**Expression and Purification of ProP-His6 and MRGSHc-ProPEc (Glu440–Glu500)–ProPEc-His6** was purified from E. coli strain WG710 (WG350 pDC79) (33) as described by Racher et al. (4).

Base pairs 1318–1500 of proPEc (encoding ProPEc (Glu440–Glu500)) were PCR amplified using primers that created flanking BamHI and HindIII restriction sites. The primers were designed to facilitate insertion of the resulting oligonucleotide in vector pQ82L (Qiagen Inc., Valencia, CA), fusing the proP-derived open reading frame to the upstream vector sequence encoding the MRGSHc tag (32). The amion and vector were cleaved, purified, mixed, and ligated, and recombinant plasmids were recovered. A primer-encoded mutation (P946T) was corrected by site-directed mutagenesis, yielding the desired plasmid (pKK2) (32), which was introduced to E. coli MG1655 (35) to create E. coli WG864.

To produce peptide MRGSHc-ProPEc (Glu440–Glu500) E. coli WG864 was cultivated in LB medium supplemented with ampicillin (100 μg/ml). Isopropyl-β-D-thiogalacto-pyranoside (final concentration 1 mM) was added at a culture A600 of 0.5–0.6, and the cells were harvested by centrifugation when the A600 reached 2. The resulting pellet was washed twice with 0.1 M potassium phosphate, pH 7.4, and resuspended in 5 ml of lysis buffer (50 mM sodium phosphate, 0.3 M NaCl, 5 mM imidazole, 1 mM Na-EDTA, pH 8) per g, wet weight. All subsequent steps were performed at 4 °C. The cells were disrupted by two passages through a French pressure cell (AMINCO, Silver Spring, MD) at 1600 bars pressure. The lysate was centrifuged in the Sorvall SS34 rotor at 12,100 × g for 20 min and then in the Beckman Ti45 rotor at 100,000 × g for 2 h. MRGSHc-ProPEc (Glu440–Glu500) was purified from the resulting supernatant by Ni2+-nitrilotriacetic acid affinity chromatography (Qiagen Inc., Valencia, CA) according to the manufacturer’s instructions for protein purification under nondenaturing conditions.

**Western Immunoblotting Analysis**—Whole cell proteins were prepared for Western Immunoblotting, and it was performed as described above (see “Transport Assays”) and by Culham et al. (18), using the procedure of Towbin et al. (37) and selective anti-ProP antibodies, prepared as follows. Anti-ProP antibodies were recovered from 4 ml of adsorbed anti-ProP serum (4) by affinity purification as described by Salamitou et al. (38) using ProP-His6 (0.3 mg) as ligand. Peptide MRGSHc-ProPEc (Glu440–Glu500) (0.6 mg) was bound to Ni2+-nitrilotriacetic acid affinity resin (0.2 ml; Qiagen Inc.) in lysis buffer containing 10 mM imidazole and no EDTA. The loaded resin was recovered in a Micro Bio-Spin chromatography column (Bio-Rad), establishing a 0.1-ml column bed. The purified anti-ProP (1 ml) was added to the column, mixed with the resin by pipetting, transferred to a 2-ml vial, and incubated at 20 °C, shaking, for 60 min. It was transferred back to the chromatography column, and the column flow-through was collected as selective anti-ProP. The recovered antibodies recognized full-length ProPEc but not MRGSHc-ProPEc (Glu440–Glu500) on a Western blot.

**Determination of Phospholipid Head Group Composition**—E. coli cells expressing ProPEc (strain WG350 pDC79) were grown as described above (see “Transport Assays”) in MOPS medium supplemented with [32P]orthophosphate at 5 μCi/ml. Polar lipids were extracted with chloroform/methanol, and thin layer chromatography was performed as described by Wikstrom et al. (39). The relative amounts of the lipid species, identified by comparison with standards, were determined with a Bio-Rad Fluor-S Multilager.

**RESULTS**

**Osmotic Adaptation of ProPEc**—We previously reported that the transport assay medium osmolality required to activate ProPEc-His6 was independent of the osmolality of the medium in which E. coli was grown (NaCl-supplemented MOPS media with osmolalities in the range 0.12–0.32 mol/kg; Fig. 1B of Ref. 10). A more complete picture emerged when the bacteria were grown at higher osmolalities (up to 0.7 mol/kg). For cells grown in the higher osmolality range, the osmolality required to activate ProPEc depended on the osmolalities of both the growth medium and the assay medium (Fig. 2).

The response of ProPEc to assay medium osmolality fits an empirical relationship (see “Experimental Procedures”) that supports extraction of parameters quantitatively describing its osmotic activation (10). Measurements of the initial rate of proline uptake (α0) as a function of osmolality (Π/RT) are used to determine the uptake rate that would be observed at infinite osmolality (Amax), the osmolality yielding half-maxi-
Tuning and Osmotic Activation of Transporter ProP

FIGURE 2. The osmolality required to activate ProP Ec increases with growth medium osmolality. E. coli strain WG350 containing pDC79 was prepared as described under “Experimental Procedures” in NaCl-free MOPS medium (0.14 mol/kg (white circles)); in the same medium adjusted with NaCl to attain osmolalities of 0.43, 0.52, 0.60, or 0.70 mol/kg (represented by increasingly dark gray circles); or in the same medium adjusted with sucrose to attain an osmolality of 0.72 mol/kg (triangles, inset). The initial rate of proline uptake via ProP Ec was measured using assay media adjusted with NaCl to the indicated osmolalities, and lines were created by regression analysis as described under “Experimental Procedures.”

FIGURE 3. Osmotic adaptation of ProP Ec, ProP At, and ProP Cg in E. coli. Parameters $\Pi_{1/2}/RT$ (the assay medium osmolality yielding half-maximal ProP activity) and $B$ (inversely proportional to the slope of the osmotic activation curve) were derived as described under “Experimental Procedures” from measurements of transporter activity in E. coli strain WG350 containing the appropriate plasmid (see “Experimental Procedures”), which had been cultivated in MOPS media with the indicated osmolalities (for primary data, see Figs. 2 and 4). Circles, ProP Ec (NaCl as osmolyte); triangles, ProP Ec (sucrose as osmolyte); squares, ProP At; diamonds, ProP Cg. Also included are data reported by Culham et al. (10) for ProP Ec cultivated in NaCl-supplemented media with osmolalities of 0.12, 0.23, and 0.32 mol/kg (TABLE ONE of Ref. 10).

Osmotic adaptation of ProP Ec would activate, cells expressing ProP Ec were grown in a sucrose-supplemented, high osmolality medium (0.72 mol/kg). A higher osmolality was required to activate ProP Ec in cells grown in this sucrose-supplemented medium than in those grown without added osmolyte (Fig. 2, inset). Furthermore, the assay medium osmolalities yielding half maximal ProP Ec activity ($\Pi_{1/2}/RT$) and the slopes of the activation curves (indicated by $B$) were similar for cells cultivated in NaCl- and sucrose-supplemented media of similar osmolalities (compare circles and triangles in Fig. 3). An osmotic adaptation process appeared to modulate the osmosensory range of ProP Ec so that its activity would vary over an osmolality range relevant to ambient conditions.

Osmotic Activation and Adaptation of ProP Orthologues—Two groups of ProP orthologues are found in bacteria, all with extended C termini (7) (Fig. 1). Group A orthologues, typified by ProP Ec, include a C-terminal $\alpha$-helical coiled-coil domain, and Group B orthologues, typified by ProP Cg, do not (Fig. 1). Putative Group A orthologue ProP At and Group B orthologue ProP Cg were expressed in E. coli, and their osmotic activation profiles were examined to determine whether the ability to undergo osmotic adaptation was correlated with the structure of the C-terminal domain.

To assess the function and osmotic sensitivity of ProP At, E. coli cells in which that transporter was expressed from plasmid vector pBAD24 were cultivated, harvested, and resuspended in low osmolality medium (0.14 mol/kg). Proline uptake rates of these bacteria increased substantially from base line levels as the assay medium osmolality approached 0.51 mol/kg (0.2 M NaCl) (data not shown), with a maximum proline uptake rate of 5.6 nmol min$^{-1}$ mg$^{-1}$ protein. This suggested an osmotic response for ProP At, although the absolute activity observed was low. Arabinose was used to induce ProP At expression so that its activity could be more directly compared with that of ProP Ec. The proline uptake rate increased with increasing arabinose concentration, as expected, and a rate of 67 nmol min$^{-1}$ mg$^{-1}$ protein (comparable with the activity of ProP Ec without arabinose induction) was achieved at an arabinose concentration of 0.4 mM (data not shown). When the impact of osmolality on ProP At activity was again determined after such arabinose induction, the resulting activity profile was similar to that of ProP Ec (Fig. 4A).

To determine whether ProP At would undergo osmotic adaptation, bacteria expressing ProP At were grown at culture osmolalities of 0.14 and 0.62 mol/kg, and proline uptake rates were measured. As for
Changes in Lipid Composition May Cause the Osmotic Adaptation of ProP—The osmolalities required to activate osmoregulated proteins BetP of *C. glutamicum* (5, 40) and OpuA of *L. lactis* (8) increase as the PG content of the membrane increases at the expense of zwitterionic lipid (phosphatidylethanolamine (PE) or phosphatidylcholine (PC)). We hypothesized that the alteration in osmotic activation threshold for the ProP orthologues might result from alterations in the anionic lipid content of the bacterial membrane due to growth in media of varying osmolalities. The phospholipid head group composition of a polar lipid extract from bacteria grown in media of increasing salinity is depicted in Fig. 1. Bacteria were cultivated in MOPS media of increasing salinity (closed symbols) and without or with sucrose (open symbols). The error bars represent the range of two independent determinations (closed symbols) or the S.D. of four replicates (open symbols). The inset illustrates the observed 2–3-fold increase in mol % of CL, the osmolality required to attain half-maximal activation of ProP (II_b/RT) in bacteria grown at the range of osmolalities indicated in A is directly correlated with their CL content. Values for II_b/RT (with asymptotic S.D. values) are derived from Fig. 3, and values for mol % CL are from A.

ProP", the osmotic activation profile for ProPat depended on the culture medium osmolality (Fig. 4, compare A and B). Furthermore, the II_b/RT values observed for ProPat (0.240 ± 0.003 and 0.346 ± 0.013 mol/kg, respectively) were similar to those for ProPec (e.g. 0.222 ± 0.005 mol/kg (growth medium osmolality 0.12 mol/kg) and 0.363 ± 0.014 mol/kg (growth medium osmolality 0.60 mol/kg)) (Fig. 3, compare squares and circles). The decrease in proline uptake activity observed at very high osmolality (over ~0.6 mol/kg) could be attributed to impaired respiration (data not shown).

Peter et al. (22) reported that ProPCg transports proline and ectoine and that it can sense and respond to osmotic changes after expression in *E. coli*. *E. coli* WG350 expressing ProPCg was grown at culture osmolalities of 0.14 and 0.60 mol/kg. As for ProPec and ProPat, ProPCg activity depended on both the growth and assay medium osmolalities (Fig. 4, compare A and B). However, the osmolalities required for half-maximal ProPCg activation (0.45 ± 0.02 and 0.56 ± 0.03 mol/kg, respectively) were up to 2-fold higher than those required to activate ProPec and ProPat, both of which include the coiled-coil domain. These observations showed that the C-terminal coiled-coil is not essential for the osmotic adaptation of ProP but did not rule out involvement of the extended, C-terminal sequences shared by all ProP orthologues (illustrated by the grey box in Fig. 1A and the sequence alignment in Fig. 1C).

Changes in Lipid Composition May Cause the Osmotic Adaptation of ProP—The osmolalities required to activate osmoregulated proteins BetP of *C. glutamicum* (5, 40) and OpuA of *L. lactis* (8) increase as the PG content of the membrane increases at the expense of zwitterionic lipid (phosphatidylethanolamine (PE) or phosphatidylcholine (PC)). We hypothesized that the alteration in osmotic activation threshold for the ProP orthologues might result from alterations in the anionic lipid content of the bacterial membrane due to growth in media of varying osmolalities. The phospholipid head group composition of a polar lipid extract from bacteria grown in media of increasing salinity is depicted in Fig. 1. Bacteria were cultivated in MOPS media of increasing salinity (closed symbols) and without or with sucrose (open symbols). The error bars represent the range of two independent determinations (closed symbols) or the S.D. of four replicates (open symbols). The inset illustrates the observed 2–3-fold increase in mol % of CL, the osmolality required to attain half-maximal activation of ProP (II_b/RT) in bacteria grown at the range of osmolalities indicated in A is directly correlated with their CL content. Values for II_b/RT (with asymptotic S.D. values) are derived from Fig. 3, and values for mol % CL are from A.
Tuning and Osmotic Activation of Transporter ProP

do not recognize epitopes present in a C-terminal ProP Ec fragment 
(Glu440–Glu500) were therefore used to determine the expression levels 
of His6-ProPEc-Δ11 and His6-ProPEc-Δ18. Western blots revealed that, 
with the specified arabinose induction, both deletion transporters and 
ProPEc were expressed to similar levels (Fig. 6B). Thus, in addition to 
altering the osmolality at which they became active, deletion of the 
C-terminal sequence that characterizes Group A ProP orthologues dra-

matically reduced the amplitude of their osmotic activation. It is pos-
bile that the deleted C-terminal sequences are also required for interac-
tions between ProPEc and amplifier protein ProQ.

DISCUSSION

ProPEc and ProPCg were previously shown to act as both osmosen-
sors and osmoregulators (4, 22). In this study, ProPAtr was found to 
function in the same manner (Fig. 4A). In addition, all three transporters 
were found to undergo osmotic adaptation (Fig. 3). Namely, the osmo-
lality required to activate each transporter was proportional to the 
osmolality of the culture medium in which the bacteria were grown. 
This adaptive phenomenon broadens the osmolality range over which 
ProP can promote bacterial osmotolerance, ensuring that the trans-
porter is poised to respond to ambient osmolality.

The osmolalities required to activate osmoregulatory transporters 
BetP of C. glutamicum and OpuA of L. lactis increase with the PG 
content of the bacterial (40) or proteoliposome (5, 8) membranes in 
which they reside. We therefore speculated that the osmotic adaptation 
of the ProP orthologues in E. coli may correlate with changes in lipid 
composition that would occur due to osmoregulation of phospholipid 
metabolism, since the cells expressing the transporters were grown in 
media with increasing osmolalities. Although effects of other param-
ters (e.g. temperature) on bacterial membrane lipid composition were 
defined prior to this study, the dependence of E. coli lipid composition 
on growth medium osmolality (and salinity) had received limited atten-
tion (41). The phospholipid composition of other bacteria is affected by 
growth medium salinity, but the physiological consequences of those 
changes are not known (42–44). In fact, the CL content of E. coli cells 
increased significantly, whereas the PE content decreased, as cells were 
grown in media with osmolalities in the range pertinent to ProPEc adap-
tation (Fig. 5). The CL content of E. coli is also known to rise during the 
transition to stationary phase (41).

In E. coli, CL (or diphosphatidylglycerol) is produced when CL syn-

thase catalyzes the condensation of two PG molecules, releasing glyc-
erol. Thus, bacteria unable to synthesize PG also lack CL (and hence 
most anionic lipid species). CL synthase activity is attributed to the 
cls gene product, but other enzymes may also contribute, since cls mutants 
contain residual CL. Transcription of cls begins immediately down-
stream from a classical σ^R regulon (45, 46) and is enhanced in station-
ary phase (47). Tropp predicted that cls expression may also be con-
trolled by stationary phase σ factor RpoS (48). Our data support that 
notion, since RpoS mediates transcription of multiple osmoregulatory 
genes including proP (49). However, cls was not identified as an RpoS-
regulated gene during multiple screens designed to identify members of 
the RpoS regulon (50); nor was it identified as responding at the tran-
scriptional level to osmotic stress (51–54).

We observed a direct relationship between the mole fraction of CL 
among E. coli polar lipids and the osmolality required to activate ProPEc 
(Fig. 5B). A causal link between changing cardiolipin content and 
altered osmoregulation of ProP activity remains to be demonstrated. 
However, the adaptation of ProP to growth osmolality could result from 
the impact of increased CL and decreased PE on bulk membrane prop-
erties (e.g. increased anionic surface charge or altered potential to form 
the nonbilayer HII phase (55)). Alternatively, ProP could interact with and 
respond directly to CL.

CL constitutes a small proportion of polar lipids in E. coli, and the 
absolute change in CL content in response to increasing osmolality is 
quite small (increase of 3–4 mol %). Polar lipid extracts from E. coli 
include phospholipids derived from both the cytoplasmic membrane 
and the inner leaflet of the outer membrane. The inner leaflet of the 
outer membrane contains a higher proportion of PE and a lower pro-
portion of PG than does the cytoplasmic membrane (56). Thus, the 
impact of increasing osmolality on the proportion of CL in the cytoplas-
mic membrane may exceed that observed for the total polar lipid pool. 
Furthermore, CL contains two negatively charged phosphate groups 
versus only one in the other phospholipids. In addition, dye-binding 

studies suggest that CL is concentrated near the septa and poles of E. coli.
cells (57). Thus, the ProPEc environment could be more strongly influenced by changing CL levels if it were similarly localized. For example, the osmolality required for ProPEc activation could be determined in part by the relative affinities of the ProPEc C terminus for itself (homodimeric coiled-coil formation) and a cytoplasmic membrane surface of varying CL content (7).

Bacteria with null mutations in cls contain less than 0.1% CL yet show few phenotypes. It is therefore likely that PG can assume many functions of CL (55). Nevertheless, certain enzymes are specifically CL-dependent (58), and CL is a structural component of certain membrane proteins, in some cases creating a deformable “cushion” between subunits (59). For example, CL is required for the formation of respiratory enzyme supercomplexes in the inner mitochondrial membrane of yeast (60, 61). An antiparallel coiled-coil structure links the subunits in ProPEc dimers within the cytoplasmic membrane of E. coli, and it is associated with activation of ProPEc at low osmolality (18) (Fig. 6). Since the osmolality required for ProPEc activation rises as the membrane CL content rises (Fig. 5), CL may intercalate between ProPEc monomers, obstructing the conformational changes necessary for transporter activation.

Our work with ProP orthologues revealed another interesting phenomenon. When grown at low osmolality, Group A ProP orthologues (ProPEc and ProPAt (Fig. 3) as well as OusA from Erwinia chrysanthemi (18), all with C-terminal coiled-coil motifs) could be activated at much lower osmolalities than a Group B orthologue (ProPCg, which lacks that motif) (see Figs. 1 and 3). Furthermore, substitution R488I, which disrupted coiled-coil formation by a peptide replica of the ProPEc C terminus, elevated the osmotic activation threshold (Fig. 6). This result corroborated our hypothesis that antiparallel coiled-coil formation by the C-terminal domains of adjacent ProPEc molecules is required for its activation at low osmolality. Although other mechanisms are possible, attenuation of ProPEc activity by these deletions may indicate that the C terminus is required for interaction of ProPEc with amplifier protein ProQ.

In addition to the structure of the C-terminal domain, the membrane lipid compositions of the bacteria that encode ProP orthologues may modulate their osmotic activation. Group B orthologue ProPCg originates in a membrane composed entirely of anionic lipid, whereas the Group A orthologues examined in this study (ProPEc and ProPAt) originate in membranes containing much less anionic lipid, as does the putative Group A orthologue from Pseudomonas putida (TABLE ONE). Indeed, even higher osmolalities were required to activate ProPAt (homodimeric coiled-coil formation) and a cytoplasmic membrane surface of varying CL content (57).

It has been proposed that osmosensing occurs when changes in cytoplasmic ionic strength or K+ concentration immediately alter interactions between existing phospholipid head groups and particular osmosensor domains or change the conformation of an osmosensory protein within the membrane (2, 7). We propose that, on a longer time scale, extracellular osmolality changes elicit physiologically relevant alterations in phospholipid head group composition. The altered membrane lipid composition may alter the structures of embedded proteins (e.g. changing the conformation of ProP in a manner that elevates the extracellular osmolality required for its activation). We therefore propose that at least some effects of phospholipid composition on ProP activation are relevant to its osmotic adaptation, not to osmosensing.

Acknowledgments—We are grateful to Doreen Callham for help with plasmid construction and to Bert Poolman and Jan Spitzer for helpful discussions of the lipid dependence of transporter activity.

REFERENCES

1. Wood, J. M., Bremer, E., Csonka, L. N., Krämer, R., Poolman, B., van der Heide, T., and Smith, L. T. (2001) Comp. Biochem. Physiol. 130, 437–460
2. Morbach, S., and Krämer, R. (2002) ChemBioChem 3, 384–397
3. Blount, P. (2003) Neuron 37, 731–744
4. Racher, K. I., Voegele, R. T., Marshall, E. V., Culham, D. E., Wood, J. M., Jung, H., Bacon, M., Cairns, M. T., Ferguson, S. M., Liang, W.-J., Henderson, P. J. F., White, G., and Hallett, F. R. (1999) Biochemistry 38, 1676–1684
5. Rübenhagen, R., Roensch, H., Jung, H., Krämer, R., and Morbach, S. (2000) J. Biol. Chem. 275, 735–741
6. van der Heide, T., and Poolman, B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7102–7106
7. Poolman, R., Spitzer, J. J., and Wood, J. M. (2004) Biochim. Biophys. Acta 1666, 88–104
8. van der Heide, T., Stuart, M. C. A., and Poolman, B. (2001) EMBO J. 20, 7022–7032
9. Krämer, R., and Morbach, S. (2004) Biochim. Biophys. Acta 1658, 31–36
10. Culham, D. E., Henderson, J., Crane, R. A., and Wood, J. M. (2003) Biochemistry 42, 410–420
11. Culham, D. E., Lasby, B., Maranongi, A. G., Milner, J. L., Steer, R. A., van Nues, R. W., and Wood, J. M. (1993) J. Mol. Biol. 229, 268–276
12. Wood, J. M., Culham, D. E., Hillar, A., Vernikovska, Ya. I., Liu, F., Boggs, J. M., and Keates, R. A. B. (2005) Biochemistry 44, 5634–5646
13. Huang, Y., Lemieux, M. J., Song, J., Auer, M., and Wang, D.-N. (2003) Science 301, 616–620
14. Hira, T., Heymann, J. A. W., Maloney, P. C., and Subramaniam, S. (2003) J. Bacteriol. 185, 1712–1718
15. Milner, J. L., and Wood, J. M. (1989) J. Bacteriol. 171, 947–951
16. Kunte, H. J., Crane, R. A., Culham, D. E., Richmond, D., and Wood, J. M. (1999) J. Bacteriol. 181, 1537–1543
17. Smith, M. N., Crane, R. A., Keates, R. A. B., and Wood, J. M. (2004) Biochemistry 43, 12997–12999
18. Culham, D. E., Tripet, B., Racher, K. I., Voegele, R. T., Hodges, R. S., and Wood, J. M. (2000) J. Mol. Recog. 13, 1–14
19. Hillar, A., Tripet, B., Zoetewe, D., Wood, J. M., Hodges, R. S., and Boggs, J. M. (2003) Biochemistry 42, 15170–15178
20. Zoetewe, D. L., Tripet, B. P., Kutateladze, T. G., Overduin, M. J., Wood, J. M., and Hodges, R. S. (2003) J. Mol. Biol. 334, 1063–1076
21. Hillar, A., Culham, D. E., Vernikovska, Ya. I., Wood, J. M., and Boggs, J. M. (2005) Biochemistry 44, 10170–10180
22. Peter, H., Weil, B., Burkovski, A., Krämer, R., and Morbach, S. (1998) J. Bacteriol. 180, 6005–6012
23. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Neidhardt, F. C., Bloch, P. L., and Smith, D. F. (1974) J. Bacteriol. 119, 736–747
25. Sambrook, J., and Russell, D. W. (2001) Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Bayliss, C., Lasby, B., Wood, J. M., Lifshitz, R., and Brown, G. L. (1993) Can. J. Microbiol. 39, 1111–1119
27. Brown, E. D., and Wood, J. M. (1992) J. Biol. Chem. 267, 13086–13092
28. Culham, D. E., Hillar, A., Henderson, J., Li, A., Vernikovska, Ya. I., Racher, K. I., Boggs, J. M., and Wood, J. M. (2003) Biochemistry 42, 11815–11823
29. Hanahan, D. (1983) J. Mol. Biol. 166, 557–569
30. Guzman, L.-M., Belin, D., Carson, M. J., and Beckwith, J. (1995) J. Bacteriol. 177, 4121–4130
31. Tsatskis, Y. (2005) The Role of the C-terminal Coiled-coil Domain in Osmosensing, Osmotic Activation, and Adaptation of Excherichia coli Transporter ProP, M.Sc. thesis, University of Guelph
32. Khamauti, J. (2003) Does the Coiled-coil Domain of ProP of Excherichia coli Mediate Osmosensing via Protein-Protein Interaction?, M.Sc. thesis, University of Guelph
33. Racher, K. I., Culham, D. E., and Wood, J. M. (2001) Biochemistry 40, 7324–7333
34. Smith, P. K., Krohn, R. L., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
Tuning and Osmotic Activation of Transporter ProP

35. Blattner, F. R., Plunkett, G., III, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D., Mau, B., and Shao, Y. (1997) Science 277, 1453–1462.
36. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
37. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354.
38. Salamitou, S., Lemaire, M., Fujino, T., Ohayon, H., Gounon, P., Beguin, P., and Aubert, J. P. (1994) J. Bacteriol. 176, 2828–2834.
39. Wikstrom, M., Xie, J., Bogdanov, M., Milejkovskaya, E., Heacock, P., Wieslander, A., and Dowhan, W. (2004) J. Biol. Chem. 279, 10484–10493.
40. Peter, H., Burkovski, A., and Kramer, R. (1996) J. Bacteriol. 178, 5229–5234.
41. Cronan, J. E. (2003) Annu. Rev. Microbiol. 57, 203–224.
42. Lopez, C. S., Heras, H., Rozal, S. M., Sanchez-Rivas, C., and Rivas, E. A. (1998) Curr. Microbiol. 36, 55–61.
43. Machado, M. C., Lopez, C. S., Heras, H., and Rivas, E. A. (2004) Arch. Biochem. Biophys. 422, 61–70.
44. Danevic, T., Rifors, L., Strancar, J., Lindblom, G., and Stopar, D. (2005) Biochim. Biophys. Acta 1712, 1–8.
45. Ivanisevic, I., Milic, M., Ajdic, D., Rakonjac, J., and Savic, D. J. (1995) J. Bacteriol. 177, 1766–1771.
46. Tropp, B. E., Ragolia, L., Xia, W., Dowhan, W., Milkman, R., Rudd, K. E., Ivanisevic, I., and Savic, D. J. (1995) J. Bacteriol. 177, 5155–5157.
47. Heber, S., and Tropp, B. E. (1991) Biochim. Biophys. Acta 1129, 1–12.
48. Tropp, B. E. (1997) Biochim. Biophys. Acta 1348, 192–200.
49. McLeod, S. M., Aiyar, S. E., Gourse, R. L., and Johnson, R. C. (2002) J. Mol. Biol. 316, 517–529.
50. Vijayakumar, S. R., Kirchhof, M. G., Patten, C. L., and Schellhorn, H. E. (2005) J. Bacteriol. 186, 8499–8507.
51. Weber, A., and Jung, K. (2002) J. Bacteriol. 184, 5502–5507.
52. Patten, C. L., Kirchhof, M. G., Schertzberg, M. R., Morton, R. A., and Schellhorn, H. E. (2004) Mol. Gen. Genet. 272, 580–591.
53. Lacour, S., and Landini, P. (2004) J. Bacteriol. 186, 7186–7195.
54. Weber, H., Polen, T., Heuveling, J., Wendisch, V. F., and Hergge, R. (2005) J. Bacteriol. 187, 1591–1603.
55. Dowhan, W. (1997) Annu. Rev. Biochem. 66, 199–232.
56. Osborn, M. J., Gander, J. E., Parisi, E., and Carson, J. (1972) J. Biol. Chem. 247, 3962–3972.
57. Milejkovskaya, E., and Dowhan, W. (2005) Curr. Opin. Microbiol. 8, 135–142.
58. Operakova, M., and Tanner, W. (2005) Biochim. Biophys. Acta 1610, 11–22.
59. Fyfe, P. K., McAuley, K. E., Rozak, A. W., Isaacs, N. W., Cogdell, R. J., and Jones, M. R. (2001) Trends Biochem. Sci. 26, 106–112.
60. Zhang, M., Milejkovskaya, E., and Dowhan, W. (2002) J. Biol. Chem. 277, 43553–43556.
61. Zhang, M., Milejkovskaya, E., and Dowhan, W. (2005) J. Biol. Chem. 280, 29403–29408.
62. Prasad, R. (1996) Manual on Membrane Lipids, p. 2, Springer, Heidelberg, Germany.
63. Karnezis, T., Fisher, H. C., Neumann, G. M., Stone, B. A., and Stanisich, V. A. (2003) J. Bacteriol. 184, 4114–4123.
64. Pinkart, H. C., and White, D. C. (2005) J. Bacteriol. 179, 4219–4226.
65. Hoinchen, C., and Kramer, R. (1990) J. Bacteriol. 172, 3409.