Outer membrane protein A of *Escherichia coli* forms temperature-sensitive channels in planar lipid bilayers

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Abstract The temperature dependence of single-channel conductance and open probability for outer membrane protein A (OmpA) of *Escherichia coli* were examined in planar lipid bilayers. OmpA formed two interconvertible conductance states, small channels, 36–140 pS, between 15 and 37°C, and large channels, 115–373 pS, between 21 and 39°C. Increasing temperatures had strong effects on open probabilities and on the ratio of large to small channels, particularly between 22 and 34°C, which effected sharp increases in average conductance. The data infer that OmpA is a flexible temperature-sensitive protein that exists as a small pore structure at lower temperatures, but refolds into a large pore at higher temperatures.

Key words: Outer membrane protein A; Channel; Planar bilayer; Temperature-sensitive; *Escherichia coli*

1. Introduction

Outer membrane protein A (OmpA) is a major protein of the *Escherichia coli* envelope that stabilizes the outer membrane [1], acts as a receptor for bacteriophages and colicins [2,3], participates in bacterial conjugation [4], mediates virulence and pathogenicity [5,6], and is an important target in the immune response [7,8]. The 325 residue mature protein consists of two domains, a 171 residue transmembrane N-terminal domain and a 154 residue periplasmic C-terminal domain linked by a Pro-Ala-rich region [9,10]. X-ray diffraction analysis of the N-terminal membrane domain, $OmpA_{171}$, determined by Pautsch and Schulz [11,12] to resolutions of 2.5 and 1.65 Å, shows that the structure consists of an eight-stranded anti-parallel β-barrel. The interior of the β-barrel, lined with charged and polar residues, encloses several unconnected aqueous cavities. The structure of the C-terminal domain is not known, but it is presumably located in the periplasmic space where it may interact specifically with the peptidoglycan layer [13,14].

Despite the lack of a continuous water channel in the crystal structure, there is now general agreement that OmpA and $OmpA_{171}$ form open pores in bilayer systems. However, ambiguities remain about the configuration of the channels formed by this monomeric protein in bilayer systems. Sugawara and Nikaido [15] found that OmpA forms diffusion channels in proteoliposomes with a pore size of 1 nm, but determined by density gradient centrifugation that only 2–3% of the OmpA population was in an open configuration. They proposed that the open and closed forms represent two alternate conformers, one that is permeable to molecules up to 600 Da and one that is essentially impermeable. Saint et al. [16,17] observed a single high conductance state in planar lipid bilayers of 180 pS for OmpA in 0.25 M KCl, and a single low conductance state of 72 pS in 1 M NaCl for $OmpA_{171}$. Arora et al. [18] noted the presence of two interconvertible conductance states for OmpA in planar lipid bilayers, low conductance channels of 50–80 pS and high conductance channels of 260–320 pS. The authors suggested that the smaller channels are associated with the N-terminal membrane domain whereas the large channels require the presence of both domains.

Nuclear magnetic resonance studies by Arora et al. [19] revealed some conformational flexibility along the axis of the β-barrel in the OmpA structure, and molecular dynamics simulations by Bond et al. [20] showed significant mobility for residues and water molecules within the β-barrel. Since molecular motions increase with temperature, we examine here the effects of temperature on the single-channel conductance and open probability of OmpA and the membrane portion of OmpA, His-tagged at the C-terminal end (His-$OmpA_{171}$), in planar lipid bilayers.

2. Materials and methods

2.1. Purification of OmpA protein

Wild-type OmpA was extracted from outer membranes of *E. coli* JM109 by modification of the method of Sugawara and Nikaido [15,21]. Briefly, stationary-phase cells were suspended in 20 mM Tris–HCl, pH 7.5, 5 mM ethylenediamine tetra-acetic acid (EDTA), 1 mM PMSF (phenylmethylsulfonyl fluoride) and disintegrated by ultrasonication (Branson). Unbroken cells were removed by centrifugation at 5000 rpm for 10 min (Sorvall GSA rotor) and crude outer membrane fractions were recovered by centrifugation at 8000 rpm for 20 min. Membranes were suspended in 0.3% lithium dodecyl sulfate (LiDS) containing 5 mM EDTA and 20 mM Tris–HCl, pH 7.5, to give a final protein concentration of 2 mg/ml. After 30 min. in an ice bath, the suspension was centrifuged at $100\,000 \times g$ for 45 min. The supernatant was discarded and the pellet was resuspended in 2% LiDS, 5 mM EDTA, 20 mM Tris–HCl, pH 7.5, and after 30 min in an ice bath the suspension was cleared by centrifugation at $100\,000 \times g$ for 45 min. The pellet was discarded and the supernatant, containing soluble OmpA, was loaded onto a column of Sephacryl S-300 (16×60, HiPrep, Pharmacia) that had been equilibrated with 0.1% LiDS, 0.4 M LiCl, 20 mM Tris–HCl, pH 7.5. Fractions were eluted

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with the same solvent and examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). OmpA-rich fractions were combined and concentrated using Centricon-10 (Amicon). The OmpA was reconstituted into liposomes of C8E4 (tetraethylene glycol monooctyl ether); Sigma Chemical) at a concentration of ca. 80 μg/ml. SDS–PAGE gels of the unheated reconstituted OmpA showed a single band corresponding to an apparent molecular weight of 30 kDa, consistent with native protein, whereas the heated protein showed a band corresponding to an apparent molecular weight of 35 kDa, consistent with denatured protein [18].

2.2. Purification of His-OmpA$_{171}$

OmpA$_{171}$, His-tagged at the C-terminal end and cloned into pBluescript KS+; Stratagene) (courtesy of R. Koebnik), was transformed into *E. coli* BL21 (Novagen) and overexpressed by addition of 1 mM IPTG. The cells were harvested by centrifugation and purified by Ni-affinity chromatography as described by the manufacturer (Qiagen).

2.3. Planar lipid bilayer measurements

Planar lipid bilayers were formed from a solution of synthetic diphytanoylphosphatidylcholine (DPhPC) (Avanti Polar Lipids) in *n*-decane (Aldrich) (17 mg/ml). The solution was used to paint a bilayer in an aperture of ~150 μm diameter between aqueous bathing solutions of 1 M KCl in 10 mM Tris, pH 7.1, in a Delrin cup (Warner Instruments). All salts were ultrapure (>99%) (Aldrich).

2.4. Temperature studies

For temperature studies, a Teflon cuvette was seated in a special outer chamber made of a polymer/graphite mixture. The chamber was fitted on a conductive stage containing a pyroelectric heater/cooler. Deionized water was circulated through this stage to remove the heat generated. The pyroelectric heating/cooling stage was driven by a temperature controller (HCC-100A, Dagan Instruments). The temperature of the bath was monitored constantly with a thermoelectric monitor in the *trans* side, i.e. the ground side, of the cuvette. Although there was a gradient of temperatures between the bath solution and conductive stage, the temperature within the bath could be reliably controlled to within ±0.5°C. After the bilayer membrane was formed, 2.5 μl of OmpA (ca. 80 μg/ml) in C8E4 micelles was added to the *cis* compartment and channels were observed. The temperature of the bath was then adjusted to the designated temperatures.

The relative change in single-channel conductance for a 10°C change in temperature, Q_{10}, was calculated from the equation:

$$Q_{10} = \left(\frac{X_2}{X_1}\right)^{10/(T_2-T_1)}$$

where X_2 is the conductance at T_2 and X_1 is the conductance at T_1 [22]. Energies of activation (E_a) were calculated from the Arrhenius equation:

$$E_a = \frac{RT_1T_2}{T_2-T_1}\ln\frac{X_2}{X_1}$$

where R is the gas constant (8.514 J/mol) and T_1 and T_2 are in K.

2.5. Recording and data analysis

Unitary currents were recorded with an integrating patch clamp amplifier (Axopatch 200A, Axon Instruments). The *cis* solution (voltage command side) was connected to the CV 201A head stage input, and the *trans* solution was held at virtual ground via a pair of matched AG-AgCl electrodes. Currents through the voltage-clamped bilayers (background conductance <6 pS) were low-pass-filtered at 10 kHz (−3 dB cutoff, Besel type response) and recorded after digitization through an analog-to-digital converter (Digidata 1322A, Axon Instruments). Using standard voltage conventions, positive clamping potentials are listed as potentials with respect to the ground (*trans* chamber), and positive currents are shown as upward deflection in the traces.

Data were filtered through an eight-pole Bessel filter (902LPF, Frequency Devices) and digitized at 1 kHz using pClamp9 software (Axon Instruments). Single-channel conductance events were identified automatically and analyzed by using Clampfit9 software (Axon Instruments). The data were averaged from 12 independent recordings by analyzing single-channel histograms for each temperature level.

3. Results

3.1. Representative single-channel recordings for OmpA in planar lipid bilayers at selected temperatures

The single-channel conductance of OmpA was determined in planar lipid bilayers at selected temperatures. OmpA, isolated from outer membranes of *E. coli* JM109 by detergent extraction and reconstituted in C8E4 micelles (see Section 2), was incorporated into bilayers of DPhPC between aqueous solutions of 1 M KCl in 10 mM Tris, pH 7.1, and maintained at the designated temperature.

Representative single-channel recordings (2 s) are shown at +100 mV in Fig. 1A for five temperature levels between 15 and 37°C (Fig. 1A). The channels were symmetric so that channel openings at negative potential were of the same amplitude (not shown). At each temperature, channel activity was observed for 20–30 min. Low and high conductance states may be discerned in the traces, referred to as small and large channels. Mean conductance of small or large channels, individually, were determined from all-point histograms (Fig. 1B). At 15°C (n = 312), OmpA displayed very short openings to small channels with a mean conductance amplitude of 30 pS. At 22°C (n = 1077), traces principally showed short openings to small channels, although brief openings to large channels were also observed; mean conductance values were 58 and 130 pS, respectively. Traces at 25.5°C (n = 519) showed more frequent and longer openings to both small and large channels with mean conductance amplitudes of 76 and 180 pS, respectively. At 30°C (n = 215), small channels were very rare and large channels were frequent and of long duration; mean conductances were 105 and 250 pS, respectively. At 37°C (n = 178), very long openings to large channels with a mean conductance of 350 pS were prevalent. All-point amplitude histograms of the two open states (Fig. 1B) clearly illustrate the transition from small (gray) to large (black) channels with increasing temperature.

Direct openings from the closed state to small channels and from the closed state to large channels, and direct closures from small channels and large channels to the closed state may be seen in traces at 15 and 22°C. Transitions from small channels to large channels and from large channels to small channels are evident in the traces at 25.5 and 30°C.

3.2. Representative single-channel recordings for His-OmpA$_{171}$ in planar lipid bilayers at selected temperatures

The single-channel conductance of the His-tagged membrane portion of OmpA, His-OmpA$_{171}$, was observed in planar lipid bilayers at selected temperatures. His-OmpA$_{171}$, overexpressed in *E. coli* BL21 by IPTG induction and purified by Ni-agarose chromatography (see Section 2), was incorporated into bilayers of DPhPC as above.

Fig. 2A shows representative single-channel recordings at −100 mV for His-OmpA$_{171}$ at four temperatures. The channels were symmetric so that channel openings at positive potentials were of the same amplitude (not shown). Channel activity was observed for 20–30 min at each temperature. Only a single conductance state was detected throughout the temperature range. No channel activity was noted at 15°C. At 22°C, openings were brief with a mean conductance of 26 pS (n = 846). With rising temperature, openings were of longer duration and conductance increased sharply. Mean conductance was 58 pS (n = 445) at 30°C, and 108 pS (n = 548) at

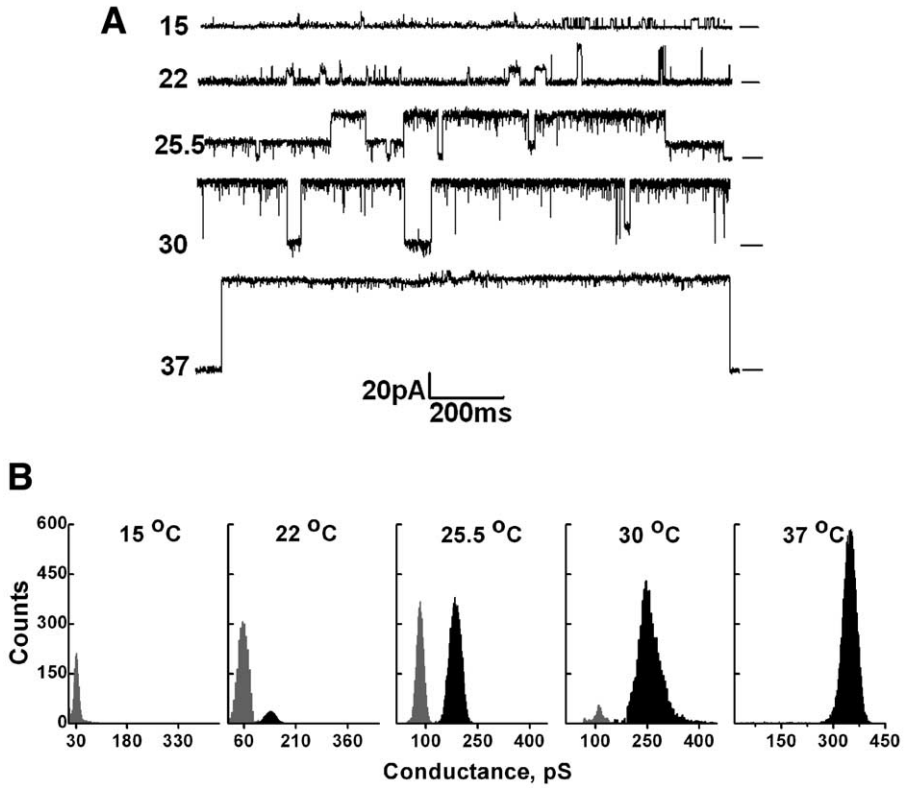

Fig. 1. A: Representative single-channel recordings for OmpA. Protein (ca. 0.2 μg) in C8E4 was incorporated in planar lipid bilayers formed from DPhPC/*n*-decane, between symmetric bathing solutions of 1 M KCl in 10 mM Tris–Cl buffer, pH 7.1. Clamping potential was +100 mV with respect to ground (*trans*). Data were filtered at 1 kHz. The temperature of the chambers was controlled by pyroelectric controller (see Section 2). The temperature in the *trans* bath (ground) was read directly using a thermoelectric junction thermometer which also served as a point of reference for the pyroelectric controller. Temperatures are indicated on the left hand side of each trace, and the closed state is delineated by a horizontal bar at the right side of each trace. B: The corresponding amplitude histograms showing the distribution of small (gray) and large (black) OmpA channels at increasing temperatures. The closed state is not shown.

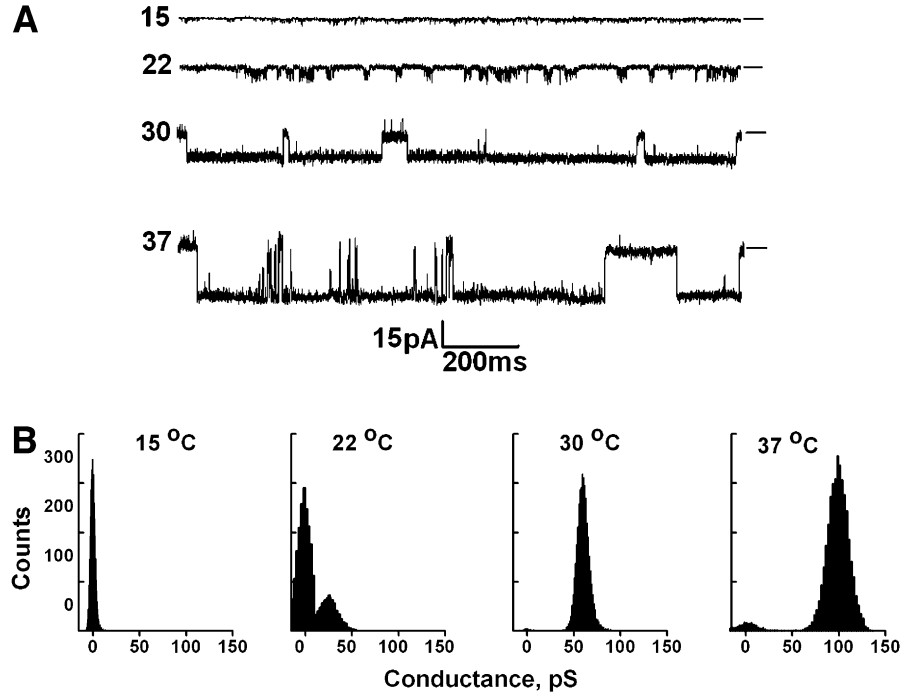

Fig. 2. A: Representative single-channel recordings for His-OmpA171. The procedure and experimental conditions are the same as described in the legend to Fig. 1. The bilayers were clamped at −100 mV, and held at the temperature indicated on the left hand side of the trace using a pyroelectric controller as in Fig. 1 (see also Section 2). The closed state is delineated by a horizontal bar at the right side of each trace. B: The associated amplitude histograms showing current distribution of conductance for His-OmpA 171.

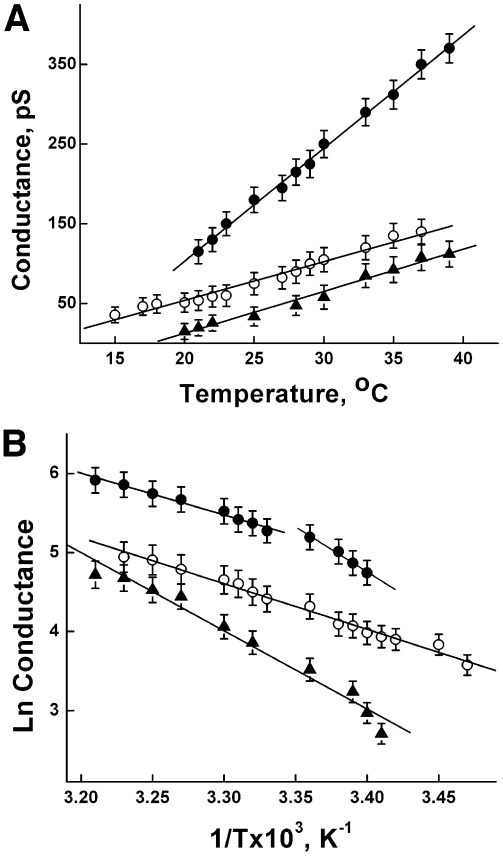

Fig. 3. A: Temperature dependence of single-channel conductance for small (○) and large (●) OmpA channels and for His-OmpA171 (▲) channels. Mean conductance values of channels were determined by analyzing a total (for all temperature levels) of 2576 small OmpA channel events, 2345 large OmpA channel events and 3207 His-OmpA171 channel events. B: Arrhenius plots of single-channel conductance for small (○) and large (●) channels of OmpA, and for His-OmpA171 (▲) channels in planar lipid bilayers.

37°C. All-point amplitude histograms in Fig. 2B illustrate the increase in conductance with increasing temperatures for the single conductance state of His-OmpA$_{171}$.

3.3. Temperature dependence of single-channel conductance for OmpA and His-OmpA$_{171}$ in planar lipid bilayers

Mean conductance values for OmpA small and large channels, individually, and His-OmpA$_{171}$ channels as a function of increasing temperature in the range of 15–39°C are shown in Fig. 3A. Above 39°C, the recordings became too erratic. Each value was determined from all-point histograms as above. Only small OmpA channels were observed below 21°C. Between 15 and 37°C, the conductance of small OmpA channels increased linearly from 36 to 140 pS. Large OmpA channels, with a conductance of 115 pS, were first observed at 21°C. Conductances of the large channels increased steeply and linearly to 370 pS at 39°C. When temperatures were decreased, the channels closed down for prolonged periods. His-OmpA$_{171}$ channels displayed the lowest conductance at all temperatures. They were closed below 20°C, and rose linearly from 15 to 112 pS between 20 and 39°C.

Arrhenius plots of single-channel conductances (Fig. 3B) indicate a linear relationship of the energy of activation (E_a) with temperature for small OmpA channels and a slightly non-linear relationship for large OmpA channels. Arrhenius plots for His-OmpA$_{171}$ channels become non-linear at low temperatures, coincident with the first opening of the channels. For purposes of discussion, Q_{10} and E_a values were calculated for the low ($\leq$21°C) and high ($\geq$35°C) temperature ranges in which small and large channels, respectively, were predominant, and the temperature range (22–34°C) in which both small and large channels were significantly present (Table 1). Q_{10}/E_a values for small and large OmpA channels were individually moderate in all three temperature ranges, suggesting that the sharp increase in conductance between 22 and 34°C was primarily due to the conversion of small channels to large channels.

3.4. Average single-channel conductance for OmpA channels in planar lipid bilayers as a function of temperature

Since the relative proportion of small and large channels changed dramatically with temperature (Fig. 1B), the weighted average for OmpA small and large channel conductance was determined at specified temperatures in the range of 15–39°C. Essentially, each value is the sum of the product of small channel amplitude and small channel open time and the product of large channel amplitude and large channel open time.

As shown in Fig. 4A, average single-channel conductance of OmpA exhibited a slightly sigmoidal dependence on temperature. Conductance increased gradually from 15 to 22°C when small channels were predominant, and then rose steeply between 22 and 35°C at an average rate of 20 pS per °C as the large channel conformation was progressively more favored. Between 22 and 34°C, the Q_{10} and E_a were the sum of the values for the small and large channels (Table 1).

The percentage of channels in the OmpA population in the large configuration ($n_L/(n_L+n_S)$) also increased in sigmoidal fashion (Fig. 4B). At lower temperatures ($\leq$21°C), large channels were not observed; they were a rapidly increasing component of the population between 22 and 35°C, and were the predominant form at temperatures $>$35°C.

3.5. Temperature dependence of open probability for OmpA and His-OmpA$_{171}$ in planar lipid bilayers

Open probabilities (P_o) were determined by statistical anal-

Table 1
The temperature coefficient (Q_{10}) and energy of activation (E_a) were calculated for the low ($\leq$21°C) and high ($\geq$35°C) temperature ranges in which small and large channels, respectively, were predominant, and the temperature range (22–34°C) in which both small and large channels were significantly present

Channel	≤21°C		22–34°C		≥35°C	
	Q_{10}	E_a (kJ/mol)	Q_{10}	E_a (kJ/mol)	Q_{10}	E_a (kJ/mol)
OmpA small	2.0 ± 0.2	44 ± 5	1.9 ± 0.2	52 ± 5		
OmpA large			2.1 ± 0.2	53 ± 6	1.5 ± 0.2	34 ± 4
Average OmpA	2.0 ± 2	44 ± 5	4.0 ± 0.4	105 ± 10	1.5 ± 0.2	34 ± 4

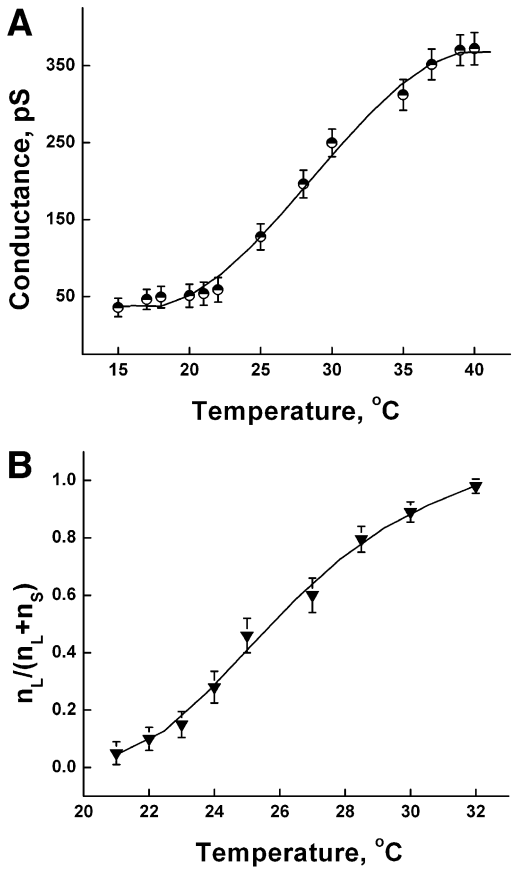

Fig. 4. A: Temperature dependence of average single-channel conductance for OmpA channels in planar lipid bilayers. Experimental conditions were as in Fig. 1. B: The proportion of large conductance channels in the OmpA population ($n_L/(n_L+n_S)$) as a function of temperature.

ysis of 3301 total events for OmpA (large and small together) and 3210 events for His-OmpA$_{171}$ at designated temperatures. Seven independent experiments were conducted for OmpA and five for His-OmpA$_{171}$ in planar bilayers under the same experimental conditions as above.

As shown in Fig. 5, the open probability of OmpA and His-OmpA$_{171}$ channels displayed a sigmoidal dependence on temperature. For OmpA, P_o increased gradually from 0.02 to 0.13 below 22°C, rose steeply between 22 and 34°C at ca 0.065 per °C, and reached a maximal value of 0.95 at 35–40°C. For His-OmpA$_{171}$, P_o was <0.02 below 22°C, rose sharply between 22 and 35°C at ca. 0.045 per °C, and leveled off to ca. 0.65 at 35–40°C.

4. Discussion

We have investigated the temperature dependence of single-channel conductance and open probability for pores formed in planar lipid bilayers by *E. coli* OmpA and the His-tagged N-terminal membrane domain of OmpA, His-OmpA$_{171}$. OmpA displayed two seemingly interconvertible open states, a low conductance state and a high conductance state, referred to as small and large channels; His-OmpA$_{171}$ exhibited only a single low conductance state. These results are in accord with Arora et al. [18], who observed low and high conductance states for OmpA and a single low conductance state for OmpA$_{171}$. We further determined that whereas temperature has only moderate effects on the single-channel conductance of small and large channels, individually, there were strong sigmoidal effects on the ratio of large to small channels and on open probability that resulted in sharp increases in average single-channel conductance of OmpA between 22 and 34°C.

The effects of temperature on individual single-channel conductances of small and large OmpA were linear (Fig. 3A), whereas average single-channel conductance of OmpA showed a sigmoidal relationship that included a steep increase between 22 and 35°C (Fig. 4A). As shown in Table 1, Q_{10}/E_a values for the small and large channels, individually, were moderate to low throughout, but values for average single-channel conductance were high in the mid-range (22–34°C), being equal to the sum of values for small and large channels, and reflecting the steep increase in average conductance (Fig. 4A) effected by the sharp increase in the proportion of large channels (Fig. 4B). The synchronous decrease in small channels and increase in large channels and the higher Q_{10}/E_a values between 22 and 34°C suggest that the large channel conformation arises from the small channel conformation.

His-OmpA$_{171}$ channels, like OmpA$_{171}$ channels [18], displayed only a single conductance state. The channels were closed below 20°C, and exhibit lower conductance than either OmpA small channels or OmpA$_{171}$ channels, which may be attributable to inhibition of ion currents by C-terminal His residues in the vicinity of the channel opening. The Q_{10}/E_a values were significantly larger than that of small and large OmpA channels between 22 and 34°C, but they reflect only small changes in low conductance amplitudes.

Open probability showed a sigmoidal relationship with temperature (Fig. 5) which correlated with changes in the ratio of large to small channels. These factors combined to effect the steep increase in average single-channel conductance between 22 and 35°C (Fig. 4A). Below 22°C, where only small channel openings were observed, conductance and P_o were very low (<50 pS; 0.02–0.13). The rapid increase in P_o between 22 and 34°C (0.065 per °C) coincides with a sharp increase in the proportion of large channels (Fig. 4B). At temperatures above 35°C, where large channels were clearly predominant, P_o reached very high values (0.95).

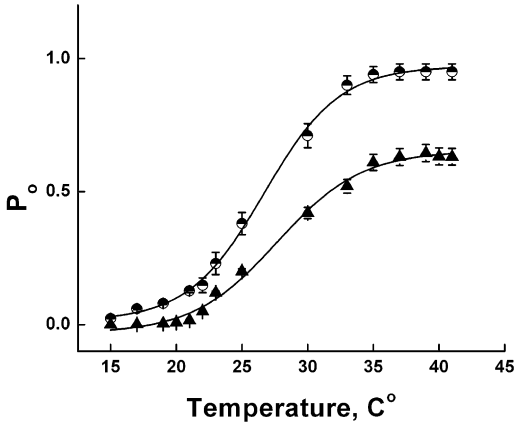

Fig. 5. Temperature dependence of open probability for OmpA (◓) (small and large channels) and His-OmpA171 (▲). Experimental conditions were as in Fig. 1. Open probability was determined statistically by analyzing 3301 events for OmpA and 3210 events for His-OmpA171 from 12 independent experiments.

Due to the steep temperature dependence of conductance, P_o, and the transition from small to large channels, small differences in bilayer temperatures can result in substantial differences in observations. The unexpectedly strong temperature sensitivity of OmpA may account for the widely held but incorrect perception of OmpA as a structural protein or small pore. With few exceptions, previous studies of OmpA pore formation were conducted at temperatures where the small conductance state of the channel is prevalent. The two conformers of OmpA in proteoliposomes noted by Sugawara and Nikaido [15] were separated by density gradient centrifugation at 10°C, hence the finding of only 2–3% of the population in the open conformation is entirely consistent with the extrapolated P_o in planar bilayers at this temperature, but not reflective of the condition of OmpA at physiological temperatures.

Our studies also support the premise put forward by Arora et al. [18] that the N-terminal membrane portion of OmpA is sufficient for formation of the small channels, whereas both domains are essential for the large channel structure. This hypothesis is also supported by studies by Saint et al. [16,17] and by the observation in this study of a single low conductance state for His-OmpA$_{171}$ (Figs. 2 and 3). Support also comes from studies of a related protein, OprF of *Pseudomonas*, which shows functional similarities to OmpA. The C-terminal domains of OprF and OmpA proteins show sequence similarity (39% identity; 56% similarity) [23]; the N-terminal domains lack substantial sequence identity, yet secondary structure predictions indicate they may also be similar. Studies in planar bilayer systems show that OprF from *Pseudomonas fluorescens* forms large channels of 250–270 pS in 1 M NaCl [24], whereas the N-terminal domain of OprF forms small channels, 75 pS in 1 M NaCl [17]. Of particular interest is a study by Dé et al. [25] that showed that the channel size of OprF in *P. fluorescens* was dependent on growth temperature. OprF from cells cultured at low (8°C) and high (28°C) temperatures produced channels with low (80–90 pS) and high (250–270 pS) conductance, respectively, in solvent-free lipid bilayers.

From these studies, one may infer that OmpA is a flexible protein that exists in two temperature-sensitive conformations. At lower temperatures, the small channel conformation is strongly favored. This is likely a two-domain configuration in which the N-terminal domain in the membrane forms a somewhat more supple pore [19,20] than depicted by the crystal structure [11,12] while the C-terminal domain remains in the periplasm. With rising temperatures, the C-terminal domain becomes more pliable, facilitating its movement into the membrane to participate in fashioning a larger β-barrel structure of 10 or more β-strands, as suggested by the computer program of Shirmer and Cowan [26] which predicts the folding patterns of outer membrane proteins. Such a structure resembles one proposed for OprF by Rawling et al. [27]. This view is also supported by studies of *Pseudomonas aeruginosa* OprF by Rawling et al. [27] and of *Salmonella enterica* OmpA (94% identical to OmpA of *E. coli*) by Singh et al. [28], which probed the cell surface of intact bacterial with monoclonal antibodies directed against the C-terminus. These authors concluded that portions of the C-terminal domain of the proteins are exposed on the bacterial surface in some cells. Although only a minor portion of the OmpA population in *S. enterica* exhibited the C-terminal residues on the cell surface, the studies were conducted at room temperatures or below where OmpA exists primarily in the two-domain configuration.

As a major outer membrane protein with $>100\,000$ copies per cell [29], OmpA is an important component of the interface between the bacterium and its surrounding environment. Accordingly, the question of whether OmpA and related proteins, such as OprF, exist as closed structural proteins or large open pores under physiological conditions is of considerable importance. Judging from its behavior in planar bilayers, one may surmise that OmpA is a flexible, temperature-responsive protein, which creates large open pores, estimated to be about 1 nm in diameter [15], in the outer membranes of *E. coli* at physiological temperatures. The pores, flanked by mobile extracellular loops, may mediate the transfer of hydrophilic molecules. This view concurs with the role of OmpA as a bacteriophage receptor and its involvement in conjugation [2,4], since it is possible that single-stranded DNA is one of the hydrophilic molecules that enters and exits the cell through the hydrophilic OmpA pore. When environmental temperatures decrease, the OmpA pore may narrow or close, thus retaining vital cell metabolites.

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