

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/11412269>

# Differential Effects of Temperature on E. coli and Synthetic Polyhydroxybutyrate/Polyphosphate Channels †

ARTICLE *in* BIOCHEMISTRY · MAY 2002

Impact Factor: 3.02 · DOI: 10.1021/bi025520g · Source: PubMed

---

CITATIONS

19

---

READS

30

3 AUTHORS, INCLUDING:



**Sangbrita Das**

Tata Institute of Fundamental Research

29 PUBLICATIONS 342 CITATIONS

SEE PROFILE



**Rosetta N. Reusch**

Michigan State University

77 PUBLICATIONS 2,052 CITATIONS

SEE PROFILE

# Differential Effects of Temperature on *E. coli* and Synthetic Polyhydroxybutyrate/Polyphosphate Channels<sup>†</sup>

S. Das,<sup>‡</sup> D. Seebach,<sup>§</sup> and R. N. Reusch<sup>\*,‡</sup>

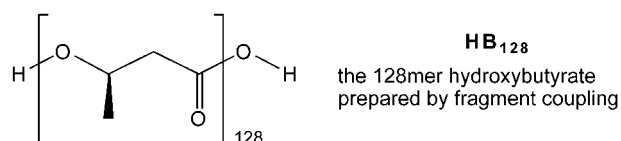
Department of Microbiology and Molecular Genetics, Michigan State University, Giltner Hall, East Lansing, Michigan 48824, and Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule Zentrum, CH-8092 Zürich, Switzerland

Received January 7, 2002; Revised Manuscript Received March 1, 2002

**ABSTRACT:** Complexes of poly-(*R*)-3-hydroxybutyrate and inorganic polyphosphate (PHB/polyP), isolated from the plasma membranes of *Escherichia coli* or prepared synthetically (HB<sub>128</sub>/polyP<sub>65</sub>), form Ca<sup>2+</sup>-selective ion channels in planar lipid bilayers that exhibit indistinguishable gating and conductance characteristics at 22 °C. Here we examine the gating and conductance of *E. coli* and synthetic PHB/polyP complexes in planar lipid bilayers as a function of temperature from 15 to 45 °C. *E. coli* PHB/polyP channels remained effectively open throughout this range, with brief closures that became more rare at higher temperatures. Conversely, as temperatures were gradually increased, the open probability of HB<sub>128</sub>/polyP<sub>65</sub> channels progressively decreased. The effect was fully reversible. Channel conductance exhibited three distinct phases. Below 25 °C, as PHB approached its glass temperature (ca. 10 °C), the conductance of both *E. coli* and synthetic channels remained at about the same level (95–105 pS). Between 25 °C and ca. 40 °C, the conductance of *E. coli* and synthetic channels increased gradually with temperature coefficients (*Q*<sub>10</sub>) of 1.45 and 1.42, respectively. Above 40 °C, *E. coli* channel conductance increased sharply, whereas the conductance of HB<sub>128</sub>/polyP<sub>65</sub> channels leveled off. The discontinuities in the temperature curves for *E. coli* channels coincide with discontinuities in thermotropic fluorescence spectra and specific growth rates of *E. coli* cells. It is postulated that *E. coli* PHB/polyP complexes are associated with membrane components that inhibit their closure at elevated temperatures.

Poly-(*R*)-3-hydroxybutyrate (PHB)<sup>1</sup> and inorganic polyphosphates (polyP) are ubiquitous constituents of biological cells (1–10). PHB, a homopolymer of (*R*)-3-hydroxybutyrate, is an amphiphilic molecule with salt-solvating properties (11–16), which derive from the ester carbonyl oxygens that recur at regular frequent intervals along its flexible backbone (17–19). PolyP, a polymorphic polyanion composed of phosphate residues linked by phosphoanhydride bonds (20), has a high capacity for ion exchange with a preference for binding multivalent cations, attributed to their higher binding energies (21).

The collective molecular characteristics and physical properties of PHB and polyP make them effective mediators of ion transport across lipid bilayers. Complexes of PHB (130–140 units) (25–27) and polyP (60–65 units) (28) have been isolated from the plasma membranes of *Escherichia coli* (22–25), and they have been prepared synthetically (26) from the 128-mer of HB (HB<sub>128</sub>) (29) and commercial polyP (average chain length 65) (26). When incorporated into planar



bilayers at physiological pH and room temperature, both *E. coli*-derived and synthetic complexes form ion channels that display the signal characteristics of protein Ca<sup>2+</sup> channels; i.e., they are selective for divalent over monovalent cations, permeant to Ca<sup>2+</sup>, Sr<sup>2+</sup>, and Ba<sup>2+</sup>, and blocked by La<sup>3+</sup> (26, 30). The effective selectivity of the channels is apparently dependent on the high negative charge density of polyP at physiological pH, since *E. coli* PHB/polyP channels become nonselective at pH 7.0 and exhibit a slight selectivity for monovalent cations at pH <7.0 (31). At physiological pH and room temperature, *E. coli* and synthetic channels have similar conductance amplitudes (104 ± 12 pS vs 101 ± 6 pS), and both display two gating modes, one mode in which there are long openings with few complete closures, and a second in which the channels open in long bursts with fast flickering closures to an intermediate closed state as well as to the completely closed state (32). The structure of the complexes is not known, but models have been suggested for the channels based on the physical properties and chain lengths of the component polymers, and the width of the bilayer. It has been proposed that Ca(polyP) traverses the bilayer surrounded and solvated by PHB, which forms either a helical structure with a lipophilic exterior of methyl groups

<sup>†</sup> Supported by NSF Grant MCB-9816053.

\* Address correspondence to this author at the Department of Microbiology and Molecular Genetics, Biomedical Physical Sciences Building, Michigan State University, East Lansing, MI 48824. Tel.: (517)355-9307; FAX: (517)353-8957; Email: mrreusch@msu.edu.

<sup>‡</sup> Michigan State University.

<sup>§</sup> Eidgenössische Technische Hochschule Zentrum.

<sup>1</sup> Abbreviations: PHB, poly-(*R*)-3-hydroxybutyrate; polyP, inorganic polyphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine.

and a hydrophilic lining of ester carbonyl oxygens (25, 26, 30), or columnar structures created by  $2_1$  helices folded in 16-mer lengths—the length of PHB chains in lamellar crystallites (33) and equal to the bilayer width (26, 34–37). Channels formed in such concentric manners will create multiple single-file pores that have a large capacity for ion transport. The efficacy of PHB and polyP in facilitating ion conduction is further demonstrated by their presence in diverse porins and channels of both prokaryotes and eukaryotes (37–40).

For practical reasons, previous planar bilayer studies of PHB/polyP channels were performed at room temperature. As stated above, the gating and conductance of *E. coli* and synthetic channels were indistinguishable in this environment (26). However, the channels normally function in *E. coli* between 30 and 40 °C, and one may expect that temperature would significantly influence the performance of ion channels formed by an amorphous polyester and polymorphic polyphosphate. In this study, we examine the effects of temperature on gating and conductance of *E. coli* PHB/polyP and synthetic HB<sub>128</sub>/polyP<sub>65</sub> channels in planar lipid bilayers. Unexpectedly, we find that the natural and synthetic channels are clearly differentiated by their divergent responses to elevated temperatures.

## EXPERIMENTAL PROCEDURES

**Media.** SOB medium (40): 2% Bacto-tryptone (Difco), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl. Transformation buffer (40): 100 mM KCl, 45 mM MnCl<sub>2</sub>, 10 mM CaCl<sub>2</sub> in 10 mM KMes, pH 6.3.

**Preparation of Competent Cells of *E. coli* DH5 $\alpha$ .** *E. coli* DH5 $\alpha$  cells were made competent by the method of Hanahan (41) as previously described (23). This procedure effects a 50–100-fold increase in the concentration of PHB/polyP complexes in *E. coli* plasma membranes. Briefly, cells were cultured in SOB medium to an absorbance of 0.4–0.6 at 550 nm. The cells were cooled, pelleted at 4 °C at low centrifugal speed (800 g), gently resuspended in one-third volume of transformation buffer at 4 °C for 30 min, and repelleted.

**Extraction of PHB/PolyP Complexes from *E. coli* Competent Cells.** PHB/polyP complexes were extracted from competent cells of *E. coli* as previously described (30). The pellet of *E. coli* competent cells was washed consecutively with methanol (2 $\times$ ), methanol/acetone (1:1), and acetone (2 $\times$ ), and the dried residue was extracted overnight at 4 °C with chloroform (10<sup>9</sup> cells/mL of CHCl<sub>3</sub>). All solvents were ice-cold and dried with Molecular Sieves (Aldrich).

**Preparation of Synthetic HB<sub>128</sub>/PolyP<sub>65</sub> Complexes.** HB<sub>128</sub> was prepared from (*R*)-hydroxybutyrate by Lengweiler et al. (29). Ca(polyP) was prepared from sodium polyphosphate glass (average residue number 65; Sigma). HB<sub>128</sub>/polyP<sub>65</sub> complexes were formed as previously described (26). Briefly, a chloroform solution of HB<sub>128</sub> (1  $\mu$ g/mL) was added to dry, finely pulverized Ca(polyP). The chloroform was removed by evaporation with a stream of dry nitrogen, and the mixture was heated in a microwave oven for 30 s (3 $\times$ ). A dry, cold chloroform solution of synthetic POPC (10  $\mu$ g/mL) was added, and the mixture was sonicated in an ultrasonication bath (47 kHz, model 2210, Branson) for ca. 20 min. The

supernatant containing the complexes was filtered with a Teflon syringe filter (0.2  $\mu$ m) and stored at –20 °C.

**Incorporation of PHB/PolyP Complexes in Lipid Bilayer Membranes.** PHB/polyP complexes, synthetic or isolated from *E. coli*, were added to a lipid mixture of synthetic POPC (Avanti Polar Lipids) and cholesterol (7:1 w/w) in decane; 1–2  $\mu$ L of a chloroform solution of the PHB/polyP complexes was added to 250  $\mu$ L of the above lipids (ratio of PHB to phospholipids was <1:1000). After removal of chloroform by evaporation with a stream of dry nitrogen, the solution was used to form lipid bilayer membranes across an aperture of  $\sim$ 150  $\mu$ m diameter in a Delrin cup (Warner Instruments). The bilayers were formed between aqueous bathing solutions of 200 mM CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub> in 10 mM KHepes, pH 7.4. All salts were ultrapure (>99%) (Aldrich).

**Temperature Studies.** For temperature studies, a special outer chamber made of polymer/graphite mixture was used in which a Teflon cuvette was seated. The chamber was fitted on a conductive stage containing a pyroelectric heater/cooler. Deionized water was circulated through this stage, fed by gravity to remove the heat generated. The pyroelectric heating/cooling stage was driven by a controller (HCC-100A, Dagan Instruments Inc.). 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 temperature between the bath solution and the conductive stage, the temperature within the bath could be reliably controlled to within  $\pm$ 0.5 °C. Once the bilayer membrane containing complexes was formed and channels were observed, the temperature of the bath was reduced to 15 °C and held constant. The bath temperature was then gradually raised to the designated temperatures.

**Recording and Data Analyses.** 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, Bessel type response) and recorded on videocassettes after digitization through an analog-to-digital converter (VR 10B, Instrutech Corp.). 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 deflections in the traces.

Data were analyzed offline after filtration through an 8 pole Bessel filter (902LPF, Frequency Devices) at 1–2 kHz as indicated in the figure legends using pClamp software (version 6.0.4, Axon Instruments) with additional standard nonlinear fitting routines where necessary. Sampling was done using a TL-1 interface (Axon Instruments) at 5–20 kHz. For constructing amplitude histograms, traces containing both the fully open and the fully closed states were selected manually. In some cases, fully closed states were extremely rare, whereas the opposite was true in others. Unitary current amplitudes were measured by fitting multiple Gaussian distributions to all point amplitude histograms. Conductance of the channel was then determined by the slope of the current–voltage curve passing through zero.

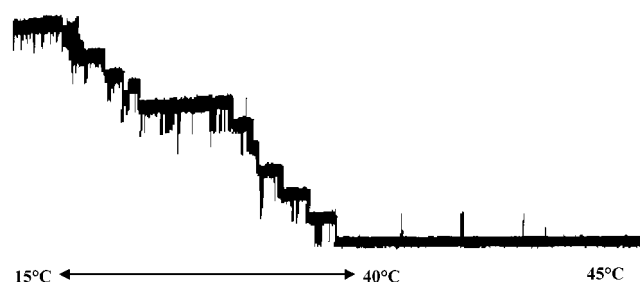


FIGURE 1: Reversible closure of synthetic HB<sub>128</sub>/polyP<sub>65</sub> channels with temperature. The synthetic complexes were incorporated into POPC/cholesterol bilayers as described in Figure 1 and under Experimental Procedures. The bilayer contained at least 11 channels. Clamping potential was +80 mV with respect to ground (trans). Data shown were filtered at 1 kHz. The temperature of the chambers was controlled by a pyroelectric controller (see Experimental Procedures). The temperature in the trans bath (ground) was directly read out with a thermoelectric junction thermometer which also served as a point of reference for the pyroelectric controller.

## RESULTS

**Effects of Temperature on Gating of *E. coli* PHB/PolyP and HB<sub>128</sub>/PolyP<sub>65</sub> Channels.** PHB/polyP complexes, extracted from genetically competent *E. coli* cells or prepared from HB<sub>128</sub> and polyP (average residue number 65) as described under Experimental Procedures, were each incorporated into planar bilayers of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC)/cholesterol (7:1) between aqueous solutions of 200 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, in 10 mM Hepes, pH 7.4. The gating of *E. coli* and synthetic channels was examined as a function of temperature in the range 15–45 °C by observing current traces during upward and downward temperature ramps. Within this temperature range, the bilayer lipids remain in the liquid-crystalline state, and PHB is above its glass temperature, estimated as ca. 10 °C (41).

The bilayer containing multiple channels (8–12) of the *E. coli* and separately of the synthetic preparations was first stabilized at a bath temperature of 15 °C using a pyroelectric heater/cooler (see Experimental Procedures). At this temperature, many, likely all, channels were open in the bilayer, and both *E. coli* and synthetic channels exhibited a large current. As the bath temperature was gradually increased to 45 °C (ca. 1 °C/min), *E. coli* channels remained open, with full closures becoming increasingly more rare (not shown). The response of the synthetic channels was very different. As temperatures were gradually increased, the number of open channels decreased progressively. By ca. 40 °C, all channels were essentially closed, and openings were infrequent and very brief. At 45 °C, channels were fully closed, and no further events were observed. A typical upward temperature ramp is shown in Figure 1. When the bath temperature was then slowly decreased to 15 °C, the number of open channels gradually increased, and all channels were open below 20 °C. The traces observed during downward temperature ramps followed essentially the same path as the upward ramps. The process was surprisingly reversible and could be repeated several times, until the bilayer membrane ruptured.

**Effect of Temperature on Single-Channel Conductance of *E. coli* PHB/PolyP and HB<sub>128</sub>/PolyP<sub>65</sub> Channels.** For these studies, *E. coli* PHB/polyP and alternatively HB<sub>128</sub>/polyP<sub>65</sub> single channels were incorporated into bilayers of POPC/cholesterol (7:1; w/w) between aqueous solutions of 200 mM

CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, in 10 mM Hepes, pH 7.4. The bath was maintained at selected temperatures in the range 22–43 °C as indicated in Figure 1, using a pyroelectrically controlled heating–cooling bath. Observations could not be made at lower or higher temperatures. Below 22 °C, channel closures were rare, and brief. Above 43 °C, current amplitudes of *E. coli* channels fluctuated widely, and channel openings of HB<sub>128</sub>/polyP<sub>65</sub> were very rare and transitory. Channel conductance was determined at each selected temperature from current–voltage relations measured at positive and negative voltages (Figure 2).

As shown in Figure 2A, the single-channel conductance of *E. coli* PHB/polyP channels remained at essentially the same level below 25 °C but, as the temperature was increased to 35 °C, single-channel conductance increased gradually with a temperature coefficient,  $Q_{10}$ , of ca. 1.45. The synthetic channels also exhibited little difference in single-channel conductance below 26 °C. From 26 to 38 °C, conductance increased with a  $Q_{10}$  of ca. 1.42 (Figure 2A). An Arrhenius plot of the data (Figure 1B) in this range indicates an energy of activation,  $E_a$ , of ca. 5 kcal/mol. Current amplitudes of the *E. coli* and synthetic channels markedly diverged above physiological temperatures. The conductance of the *E. coli* channels increased sharply, whereas the conductance of the synthetic channels leveled off.

**Representative Single-Channel Current Traces at Two Temperatures.** Long continuous single-channel current traces of *E. coli* PHB/polyP channels and HB<sub>128</sub>/polyP<sub>65</sub> channels in bilayers of POPC/cholesterol (10:1) between aqueous solutions of 200 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, in 10 mM Hepes, pH 7.4, are shown at two selected temperatures in Figure 3 A,D. Figure 3A shows 18 s of current traces for the *E. coli* channels at 25 and 40 °C. Figure 3D shows 200 s of current traces for HB<sub>128</sub>/polyP<sub>65</sub> channels at 24 and 40 °C. An expanded 1.5 s of the current records and the corresponding all-points histograms are shown for *E. coli* (Figure 3B,C) and synthetic channels (Figure 3E,F).

The traces for *E. coli* channels at 25 °C are similar to those of synthetic channels at 24 °C, though full closures are more frequent for the *E. coli* channels. However, there is a striking difference in the current traces for the two channels at 40 °C. The *E. coli* channels are largely open, they show a wider amplitude distribution, and complete closures are relatively infrequent. Conversely, the synthetic channels are mainly closed with rare sporadic openings.

## DISCUSSION

In this study, we find that Ca<sup>2+</sup> channels formed by *E. coli* PHB/polyP complexes and synthetic HB<sub>128</sub>/polyP<sub>65</sub> complexes in planar lipid bilayers can be differentiated by their divergent responses to temperatures near and above physiological levels. This is the first significant difference discerned in the performance of ion channels formed by the natural and synthetic complexes.

PHB/polyP channels are fundamentally polymer electrolyte complexes spanning a lipid bilayer. Due to its amphiphilic nature, PHB has the capacity to solvate polyP salts within a hydrophobic environment. PHB has the characteristic molecular structure of salt-solvating polymers, i.e., a flexible backbone bearing electron-coordinating groups at suitable intervals along the backbone to replace the hydration shell



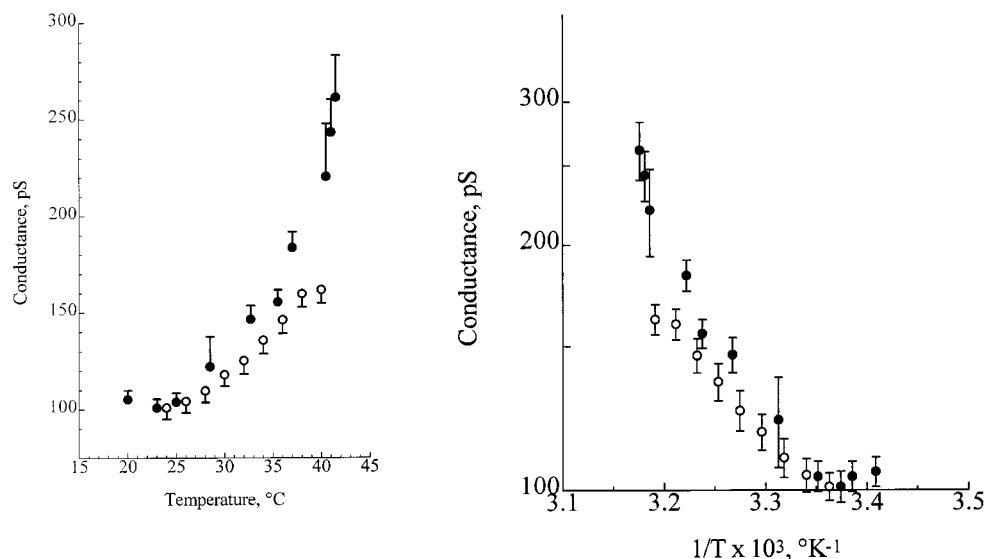


FIGURE 2: (A) Effect of temperature on conductance of *E. coli* and synthetic HB<sub>128</sub>/polyP<sub>65</sub> channels. Channels were each incorporated in bilayers of POPC/cholesterol (7:1) between symmetric aqueous solutions of 200 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 10 mM Hepes, pH 7.4. Currents were measured at positive and negative potentials. Synthetic HB<sub>128</sub>/polyP<sub>65</sub> channels (○) and *E. coli* PHB/polyP channels (●) were each incorporated into planar lipid bilayers composed of synthetic 1-palmitoyl-2-oleoylphosphatidylcholine and cholesterol (10:1 w/w) between symmetric solutions of 200 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 10 mM Tris–Hepes, pH 7.4. The temperature of the chambers was controlled by a pyroelectric controller (HCC-100A, Dagan Instruments Inc.). Channel conductance at each temperature was determined from current–voltage relations (see Experimental Procedures). The graph shows fairly similar behavior of both the synthetic and natural PHB/polyP channels up to physiological temperatures, but dramatically opposite behavior above physiological temperatures. Synthetic channels were closed, and *E. coli* channels were open. (B) Arrhenius plot of data from (A). Conductance of HB<sub>128</sub>/polyP<sub>65</sub> (○) and *E. coli* PHB/polyP (●) complexes as a function of temperature. The log of conductance is plotted against the inverse of absolute temperature (K).

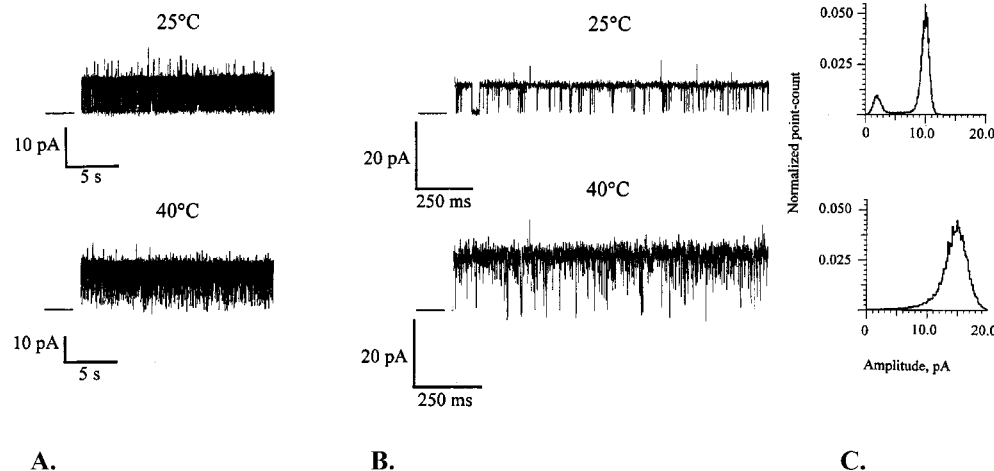
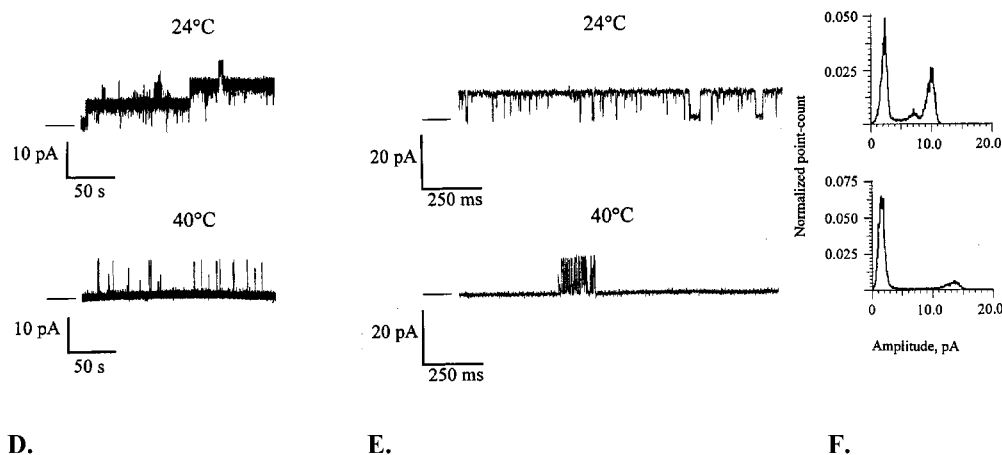
of cations (17–19). The extremely high conformational flexibility of the PHB backbone has been amply demonstrated by circular dichroism and fluorescence (FRET) measurements (36), NMR spectroscopy of isotopically labeled OHBs (43), molecular dynamics simulations of OHB hexamers (44), and NMR analysis of  $\beta$ -depsides (oligo-3-alkanoic acid derivatives with alanine, valine, and leucine side chains) (45). The salt-solvating properties of PHB have been well-established by crystal structures of salts of cyclic triesters of (*R*)-3-hydroxybutyrate (triolides) (11), and by studies that demonstrate the ability of oligo-HBs to transport cations across methylene chloride layers in U-tubes (12), poly-(*R*)-3-hydroxyalkanoates to form ion-conducting complexes with lithium perchlorate (14), and oligo-HBs to form large-conducting nonselective ion channels in planar lipid bilayers (13) and liposomes (15). In polymer electrolytes, cation transport is effected by segmental motions of the polymer backbone that carry the cations from one binding site to the next (17–19). Accordingly, conductance occurs only above the glass temperature of the polymer, and conductance amplitude increases directly with temperature.

In PHB/polyP channels, it is postulated that the polyanion, polyP, forms a ladder of closely spaced, negatively charged binding sites, traversing the bilayer, and fashioning the core of the channel. PHB forms a helical or  $\beta$ -sheet columnar structure that surrounds the polyP salt, and the ester carbonyl oxygens of PHB and the phosphoryl oxygens of polyP create binding cavities with a ligand geometry particularly suitable for binding calcium ions (25, 26, 30, 33–36, 46, 47). In effect, calcium ions bridge the polyP and PHB polymers. When a voltage pulse or concentration gradient is applied, calcium ions flow along the polyP track aided by segmental motions of the PHB backbone and libratory motions of the ester oxygens. One may predict from this hypothetical

structure that PHB/polyP channels would be functional above the glass temperature of PHB, and that the conductance amplitude would increase directly with temperature. It should be noted that the situation is made somewhat more complex by the bilayer lipids surrounding PHB, since they also may influence segmental motions of its backbone.

The responses of *E. coli* and synthetic channels to gradual temperature increases from 15 to 45 °C are completely reversible, indicating that the complexes retain their structural integrity throughout this range. The gating and conductance of the *E. coli* and synthetic channels are nearly indistinguishable at room temperature, where all previous studies were conducted, and below room temperature, but surprisingly they react to more elevated temperatures in divergent manners. As temperatures rise, *E. coli* channels remain open, and closures become progressively more brief and rare (Figure 3A). Conversely, HB<sub>128</sub>/polyP<sub>65</sub> tends to shut down; closures become more frequent and are of longer duration (Figure 3B). The stepwise closure of HB<sub>128</sub>/polyP<sub>65</sub> channels at increasing temperatures is particularly interesting (Figure 1). With rising temperature, the proportion of open channels decreases, and all channels are essentially closed above 40 °C. Channel closure may signify obstruction of ion flow by increased backbone motions of PHB. When temperature decreases, the channels progressively reopen.

The conductance of *E. coli* PHB/polyP and HB<sub>128</sub>/polyP<sub>65</sub> channels in bilayers of POPC/cholesterol (7:1) follows the predicted behavior at temperatures up to 37 °C (Figure 2). Below room temperature, as the glass temperature of PHB is approached, the channels appear to stiffen. Full closures are rare, making current amplitudes difficult to measure, but they remain approximately in the same range. Between room temperature and physiological temperature, both *E. coli* and HB<sub>128</sub>/polyP<sub>65</sub> channels display the expected increase in

***E. coli* PHB/polyP****HB<sub>128</sub>/polyP<sub>65</sub>**

**FIGURE 3:** Current traces of *E. coli* PHB/polyP channels and synthetic HB<sub>128</sub>/polyP<sub>65</sub> channels at two temperatures. The complexes were each incorporated in POPC/cholesterol bilayers as described in Figure 1 and under Experimental Procedures. (A) shows 18 s continuous current traces for *E. coli* PHB/polyP, and (D) shows 200 s continuous current traces for HB<sub>128</sub>/polyP<sub>65</sub> channels at the indicated temperatures. Note the difference in current scale for the recording of the *E. coli* channel at 40 °C. The bilayers were clamped at +80 mV, and were held at the indicated temperatures using a pyroelectric controller as in Figure 1 (see also Experimental Procedures). The closed state is indicated by a horizontal bar at the left of each trace. (B) and (E) show 1.5 s current traces at the indicated temperatures under identical conditions. The amplitude histograms (C and F) at the right were constructed from manually selected stretches of current records.

conductance with temperature (Figure 2) with comparable temperature coefficients,  $Q_{10}$ , of 1.45 and 1.42, a rate that correlates with an  $E_a$  of ca. 5 kcal/mol, close to that expected for free diffusion. Above 40 °C, the conductance of synthetic channels, in their rare and brief openings, remains at approximately the same level, whereas *E. coli* channels display sharp increases in conductance with wide fluctuations.

The sharp increase and wide variation in conductance of *E. coli* channels above 40 °C suggest that the channels are becoming disorganized. Interestingly, this event coincides with a discontinuity in thermotropic fluorescence spectra observed in membranes of genetically competent *E. coli* cells (23, 25), using the hydrophobic fluorescence probe *N*-phenyl-1-naphthylamine (NPN). It should be noted that the fluorescence spectra are observed in genetically competent cells because the concentration of PHB/polyP complexes in the membranes is 50–100-fold higher than in log-phase cells (23). As temperature is gradually increased above room

temperature, the fluorescence of NPN first decreases gradually with increasing temperature, signifying a steady decrease in membrane viscosity, and then exhibits a sharp increase in intensity from ca. 40 to 56 °C that is attributed to increased membrane viscosity as the complexes become disorganized and dissociated. The fluorescence spectra are irreversible, indicating that the complexes suffer complete dissociation at temperatures  $\geq 56$  °C; however, the data here suggest that many if not all the channels can withstand temperatures up to 45 °C, at least for several minutes. Conductance amplitudes could not be measured at temperatures above 45 °C due to system instability. It is also of interest that temperatures at which discontinuities occur in Arrhenius plots of *E. coli* channel conductance (Figure 2), i.e., below room temperature and above physiological temperature, correlate closely with temperatures at which discontinuities have been noted in Arrhenius plots for *E. coli* specific growth rates (48). This coincidence and the concurrence of the upper temperature discontinuity with the thermotropic fluorescence

curves of *E. coli* cells suggest that the channels may have a significant physiological role.

The failure of the *E. coli* PHB/polyP channels to close at elevated temperatures (Figures 2 and 3) suggests that the complexes are shielded from the bilayer by other less flexible membrane components, most likely proteins. In this regard, it is notable that extracts of PHB/polyP complexes from *E. coli* contain several proteins (30). These proteins were regarded to be nonassociated or only loosely associated with the complexes, since they can be separated from them by nonaqueous size-exclusion chromatography. The PHB/Ca-(polyP) complexes elute from the column in a fraction corresponding to molecular mass of 17–20 kDa. The proteins, however, are excluded from the column; they remain at the column entry and may be recovered by backwash. The 'protein-free' PHB/polyP complexes are very labile, but at physiological pH and room temperature, current traces obtained in planar bilayers closely resemble those of the PHB/polyP/protein co-extracts, demonstrating that the co-extracted proteins are not essential for normal channel function, at least under these conditions.

The results of our studies, showing higher stability of native versus synthetic PHB/polyP complexes, may be rationalized by assuming that one or more of the co-extracted proteins surround the complexes in the bilayer. The enveloping protein(s) may absorb thermal energy, and hydrophobic attractions between the methyl groups of PHB and hydrophobic amino acids of the associated protein(s) may restrain backbone segmental motions, thus inhibiting channel closure. In this manner, protein may moderate the effects of increasing temperature and preclude channel closure in this critical physiological range. The synthetic complexes, without the benefit or interference of proteinaceous associates, respond more sensitively and directly to this environmental change.

## REFERENCES

- Reusch, R. N. (1989) *Proc. Soc. Exp. Biol. Med.* 191, 377–381.
- Reusch, R. N. (1992) *FEMS Microbiol. Rev.* 103, 119–130.
- Reusch, R. N., Sparrow, A. W., and Gardiner, J. (1992) *Biochim. Biophys. Acta* 1123, 33–40.
- Seebach, D., Brunner, A., Bürger, H. M., Schneider, J., and Reusch, R. N. (1994) *Eur. J. Biochem.* 224, 317–328.
- Müller, H. M., and Seebach, D. (1993) *Angew. Chem., Int. Ed. Engl.* 32, 157–249.
- Reusch, R. N. (1999) *Prog. Mol. Subcell. Biol.* 23, 1–18.
- Kornberg, A. (1995) *J. Bacteriol.* 177, 491–495.
- Kornberg, A. (1999) *Prog. Mol. Subcell. Biol.* 23, 1–18.
- Kulaev, I. S. (1979) *The Biochemistry of Inorganic Polyphosphates* (Brookes, R. F., Ed.) Wiley, New York.
- Kulaev, I. S., Kulakovskaya, T. V., Andreeva, N. A., and Lichko, I. P. (1999) *Prog. Mol. Subcell. Biol.* 23, 27–43.
- Seebach, D., Bürger, M., Plattner, D. A., Nesper, R., and Fässler, T. (1993) *Helv. Chim. Acta* 76, 2581–2601.
- Bürger, H. M., and Seebach, D. (1993) *Helv. Chim. Acta* 76, 2570–2580.
- Seebach, D., Brunner, A., Bürger, H. M., Reusch, R. N., and Bramble, L. L. (1996) *Helv. Chim. Acta* 79, 507–517.
- Reusch, R. N., and Reusch, W. H. (1996) U.S. Patent No. 5,266,422.
- Fritz, M. G., Walde, P., and Seebach, D. (1999) *Macromolecules* 32, 574–580.
- Das, D., Kurcok, P., Jedlinsky, Z., and Reusch, R. N. (1999) *Macromolecules* 32, 8781–8785.
- Armand, M. B. (1987) in *Polymer Electrolyte Reviews-1* (MacCallum, J. R., and Vincent, C. A., Eds.) pp 1–37, Elsevier Applied Science, New York.
- MacCallum, J. R., and Vincent, C. A. (1987) in *Polymer Electrolytes Review-1* (MacCallum, J. R., and Vincent, C. A., Eds.) pp 23–37, Elsevier, London.
- Gray, F. M. (1992) *Solid Polymer Electrolytes*, pp 1–4, VCH, New York.
- Corbridge, D. E. C. (1985) Phosphorus. An outline of its Chemistry, Biochemistry and Technology. *Stud. Inorg. Chem.* 6, 170–178.
- Majling, J., and Hanic, F. (1980) *Top. Phosphorus Chem.* 10, 341–502.
- Reusch, R. N., and Sadoff, H. L. (1983) *J. Bacteriol.* 156, 778–788.
- Reusch, R. N., Hiske, T. W., and Sadoff, H. L. (1986) *J. Bacteriol.* 168, 553–562.
- Reusch, R., Hiske, T., Sadoff, H., Harris, R., and Beveridge, T. (1987) *Can. J. Microbiol.* 33, 435–444.
- Reusch, R. N., and Sadoff, H. L. (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85, 4176–4180.
- Das, S., Lengweiler, U. D., Seebach, D., and Reusch, R. N. (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94, 9075–9079.
- Seebach, D., Brunner, A., Bürger, H. M., Schneider, J., and Reusch, R. N. (1994) *Eur. J. Biochem.* 224, 317–328.
- Castuma, C., Huang, R., Kornberg, A., and Reusch, R. N. (1995) *J. Biol. Chem.* 270, 12980–12983.
- Lengweiler, U. D., Fritz, M. G., and Seebach, D. (1996) *Helv. Chim. Acta* 79, 670–701.
- Reusch, R. N., Huang, R., and Bramble, L. L. (1995) *Biophys. J.* 69, 754–766.
- Das, S., and Reusch, R. N. (2001) *Biochem.* 40, 8781–8786.
- Das, S., and Reusch, R. N. (1999) *J. Membr. Biol.* 170, 135–145.
- Seebach, D., Bürger, H. M., Müller, H. M., Lengweiler, U. D., and Beck, A. K. (1994) *Helv. Chim. Acta* 77, 1099–1123.
- Seebach, D., Brunner, A., Bachmann, B., Hoffmann, T., Kühnle, F. N., and Lengweiler, U. D. (1996) *Ernst Shering Res. Found.* 28, 1–105.
- Seebach, D., and Fritz, M. G. (1999) *Int. J. Biol. Macromol.* 25, 217–236.
- Rueping, M., Dietrich, A., Buschmann, V., Fritz, M. G., Sauer, M., and Seebach, D. (2001) *Macromolecules* 34, 7042–7048.
- Reusch, R. N., Huang, R., and Kosk-Kosicka, D. (1997) *FEBS Lett.* 412, 592–596.
- Reusch, R. N. (1999) *Biochemistry* 38, 15666–15672.
- Gardioli, A., Shabalin, Y., and Reusch, R. N. (1999) *Abstr. Biophys. Soc.*, A286.
- Das, S., Ghosh, P., Bera, A. K., and Reusch, R. N. (1999) *Abstr. Biophys. Soc.*, A438.
- Hanahan, D. (1983) *J. Mol. Biol.* 166, 557–580.
- Holmes, P. A. (1987) in *Developments in Crystalline Polymers* (Bassett, D. C., Ed.) pp 1–65, Elsevier, New York.
- Waser, P., Rueping, M., Seebach, D., Duchardt, E., and Schwalbe, H. (2001) *Helv. Chim. Acta* 84, 1821–1845.
- Gee, P. J., Hamprecht, F. A., Schuler, L. D., vanGunsteren, W. F., Duchardt, E., Schwalbe, H. A., Albert, M., and Seebach, D. (2002) *Helv. Chim. Acta* 85 (in press).
- Albert, M., Seebach, D., Duchardt, E., and Schwalbe, H. (2002) *Helv. Chim. Acta* 85 (in press).
- Reusch, R. N. (2000) in *Advances in Supramolecular Chemistry* (Gokel, G. W., Ed.) Vol. 7, pp 151–182, JAI Press Inc., Stamford, CT.
- Reusch, R. N. (2000) *Biochemistry (Moscow)* 65, 280–295.
- Ingraham, J. L., and Marr, A. C. G. in *Escherichia coli and Salmonella* (Neidhardt, F. C., Ed.) pp 1570–1578, ASM Press, Washington, DC.