

# Native and chemically modified porin channels from *Salmonella typhi* Ty2 in planar lipid bilayers

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Native porins, from *Salmonella typhi* Ty2 outer membrane, and porins alkylated with pyridoxal phosphate (Plp) were studied in planar lipid bilayers. The conductance of bilayers exposed to native or chemically modified porins increases in discrete jumps. Conductance histograms for native porins displayed two major peaks at 1.7 and 6.7 nS (in 0.5 M KCl). On the other hand, Plp-treated porins exhibited a single major peak at 1 nS. The relation between bilayer conductance and native porin concentration was linear. However, this relation became logarithmic in the presence of modified porins. The results support the notion that alkaline reduction of *S. typhi* Ty2 porins with Plp dissociates porin channel trimers in a reversible fashion.

(*Salmonella typhi* Ty2) Porin Pyridoxal phosphate Planar bilayer

## 1. INTRODUCTION

Porins, from the outer membrane of several gram-negative bacteria, form large permeability channels in model bilayers [1]. Ions and molecules as large as 500 Da can permeate these transmembrane channels [2-4]. Unitary conductances, estimated in planar lipid bilayers, range from 1 to 5 nS, in 1 M KCl [4-6]. The experimental evidence indicates that a porin channel results from the association of 3 monomers [5,7,8].

Recently, Calderón et al. [9] reported the isolation of two porin species from the outer membrane of *Salmonella typhi* Ty2 OmpC and OmpF with apparent molecular masses of 35 and 36 kDa respectively. These porins were found to induce the lysis of red blood cells. Furthermore, they reported that reductive alkylation of *S. typhi* Ty2 porins with pyridoxal phosphate (Plp) rendered them soluble in aqueous solution. However, the modified porins retained their ability to lyse red blood cells [9]. These observations led us to perform experiments directed at establishing the possible channel-forming properties of native and

chemically modified porins from *S. typhi* Ty2 in planar lipid bilayers. Here, we offer evidence that both native and modified porins induce increases in the conductance of planar bilayers. We found, however, that porins treated with Plp differ from native ones in two respects: the dependence of the bilayer conductance on the concentration of modified porins is logarithmic rather than linear; and the average size of the discrete conductance jump induced by modified material is only 58% that of the native porins.

## 2. MATERIALS AND METHODS

*S. typhi* Ty2 was from our strain collection. Bacteria were grown in Carlquist ninhydrin base medium (Fisher) with 1.5% agar. Outer membranes were obtained according to Moore et al. [10]. OmpC and OmpF were purified as described in [9]. Porins were identified in SDS-polyacrylamide gel electrophoresis, according to Ames [11]. The purified oligomers, suspended in 200 mM Tris-HCl, pH 7.5, were alkylated with 10 mM Plp and reduced with 0.5 mg/ml of sodium

borohydride [12]. Protein concentration was measured according to Markwell et al. [13]. SDS was estimated following Hayashi [14].

Planar lipid bilayers were formed as in [16]. Briefly, a small amount of oxidized cholesterol (20 mg/ml, in *n*-decane) was applied to a hole drilled in a polystyrene partition separating two aqueous chambers. In all experiments the buffer consisted of 0.5 M KCl, 5 mM Hepes, pH 7.0. Bilayer thinning was monitored optically and through capacitance measurements. Porins were added into the *cis* chamber and the *trans* chamber was defined as zero voltage. Current was measured and voltage applied through Ag/AgCl electrodes. The *trans* side of the bilayer was connected to the input of a variable-gain current-voltage transducer. The output was recorded on a storage oscilloscope and chart recorder. Experimental records were analyzed by hand. Electrical conductance is expressed in units of nS.

### 3. RESULTS

#### 3.1. Macroscopic conductance measurements

##### 3.1.1. Native porins

Addition of native porins from *S. typhi* Ty2 to the solution bathing the planar lipid bilayer induced large, step-wise increases in conductance ( $G_m$ ). Fig. 1A shows an experimental record illustrating this effect. The magnitude of  $G_m$  induced by a given porin concentration varied from membrane to membrane. However, in the same bilayer,  $G_m$  estimated 10–15 min after porin addition was proportional to porin concentration in the aqueous solution. As shown in fig. 1B,  $G_m$  is a linear function of porin concentration ( $P$ ). The inset to fig. 1B shows a double-logarithmic plot of  $G_m$  vs  $P$ . The experimental points are joined by a straight line. The best fit yielded a slope of 1.1 ( $r^2=0.99$ ). In 4 additional experiments, over the same concentration range, the slope averaged  $0.98 \pm 0.10$  (SE).

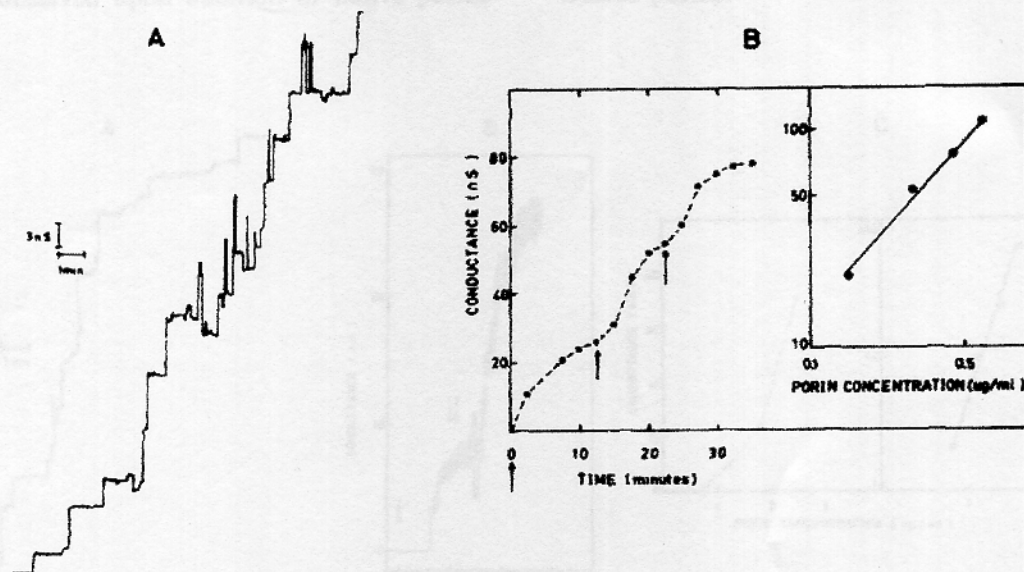


Fig. 1. Increases in bilayer conductance induced by native porins. Porins were added into the *cis* membrane chamber. In all cases the aqueous phase consisted of 0.5 M KCl, 5 mM Hepes, pH 7.0, and the applied voltage was 20 mV. SDS in the *cis* chamber never exceeded  $10^{-6}$  M. (A) Experimental record showing stepwise conductance increase induced by 0.5  $\mu$ g/ml (final concentration) native porins. (B) Bilayer conductance measured at several time points and at 3 porin concentrations (0.15, 0.3, 0.45  $\mu$ g/ml, final concentration). Arrows indicate times of porin addition. Inset: double-logarithmic plot of  $G_m$  vs  $P$ .



### 3.1.2. Modified porins

Porins modified with Plp were also active in inducing step-wise increases in  $G_m$ . This is illustrated in fig.1A. Furthermore, the relation between  $G_m$  and modified porin concentration was found to be supralinear, rather than linear. This finding is documented in fig.2B and C. As shown in fig.2B, the conductance of a bilayer after addition of 0.15 mg (final concentration) modified porin (indicated by the single arrow) reaches a value of some 7 nS, several minutes after addition. When this concentration is doubled (double arrows), the conductance rises to close to 27 nS, almost 3-times the increase induced by the previous concentration. Fig. 2C is a plot of  $G_m$  vs  $P$  over a wider concentration range of modified porins. The inset to fig.2C is a double-logarithmic plot of the same data. The slope of the best linear fit yielded a value of 2.9 ( $r^2=0.98$ ). In 4 additional studies the slope was  $2.8 \pm 0.15$  (SE).

## 3.2 Conductance-jump size distribution

### 3.2.1. Native porins

As illustrated in fig.1A, the conductance increases observed upon addition of native porins

occur in discrete jumps. Fig.3A displays a histogram of these jumps. It was constructed by pooling together data from several membranes. We observe the occurrence of two major peaks, centered at about 1.7 and 6.7 nS. Fig.3B documents the occurrence in experimental records of large fluctuations in bilayer conductance, resembling the opening and closing of single channel units, with conductances in the 6 nS range. Note also the occurrence of fluctuations in the open state of these large units, having the appearance of substates of conductance.

### 3.2.2. Modified porins

Figs 2A and 4A display experimental records of conductance fluctuations induced by Plp-treated porins. Clearly, these fluctuations are smaller than those induced by native material. Fig.4B offers a conductance histogram derived from records similar to those shown in fig.4A. We find that most of the discrete conductance jumps now lie in the 0.5–1.5 nS region. Moreover, the peak at 6.7 nS is not present here. Table 1 lists the average size of conductance jumps obtained for native and Plp-treated porins.

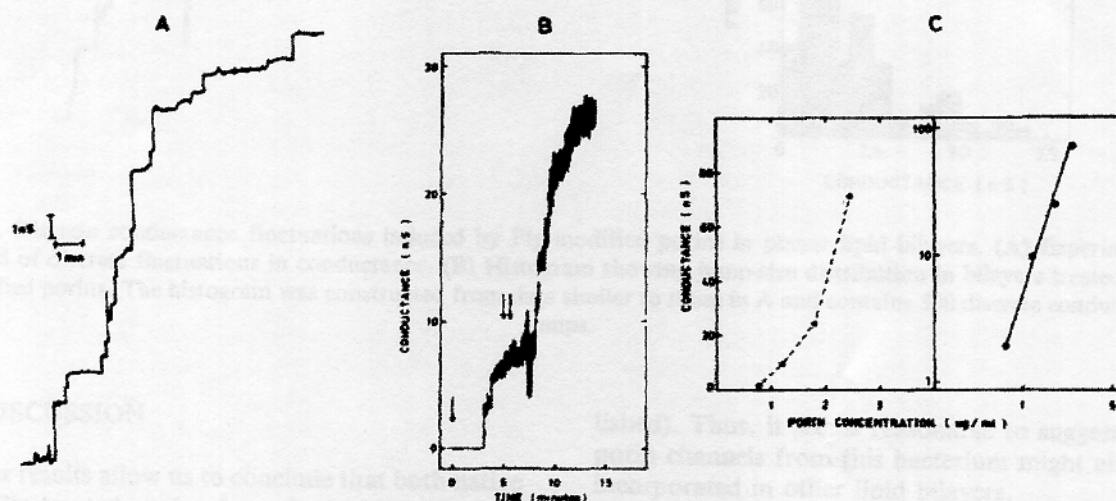


Fig.2.  $G_m$  as a function of Plp-treated porin concentration. (A) Conductance increases induced by Plp-treated porins. Final porin concentration was 0.5  $\mu\text{g/ml}$ . (B) Experimental record showing  $G_m$  at two porin concentrations. The first arrow indicates the addition of 0.15  $\mu\text{g/ml}$  porin (final concentration) into the *cis* chamber. At the time indicated by the double arrow, the concentration was doubled to 0.3  $\mu\text{g/ml}$ . (C)  $G_m$  vs concentration of modified porin. Inset: double-logarithmic plot of data shown in B.

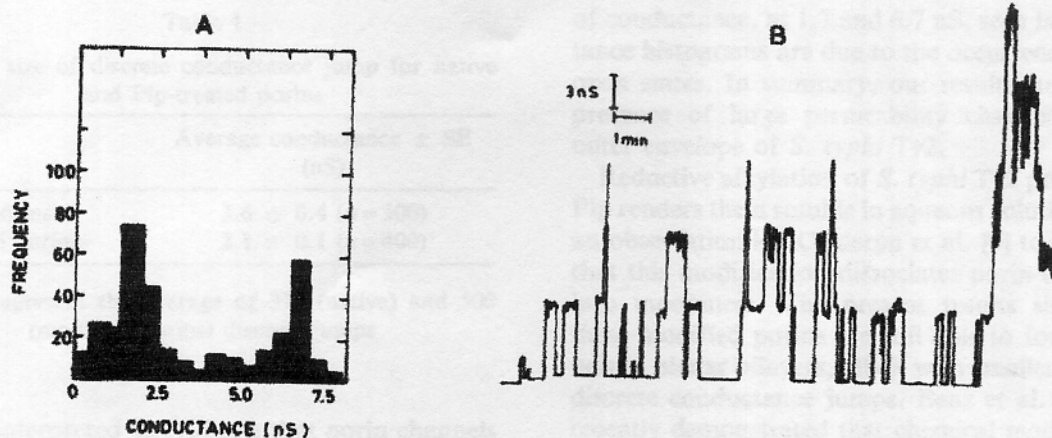


Fig.3. Size of discrete conductance jumps induced by native porins. (A) Conductance histogram showing jump-size distribution. The number of discrete jumps used to construct the histogram was 370. (B) Experimental record of large, single channel-like conductance fluctuations.

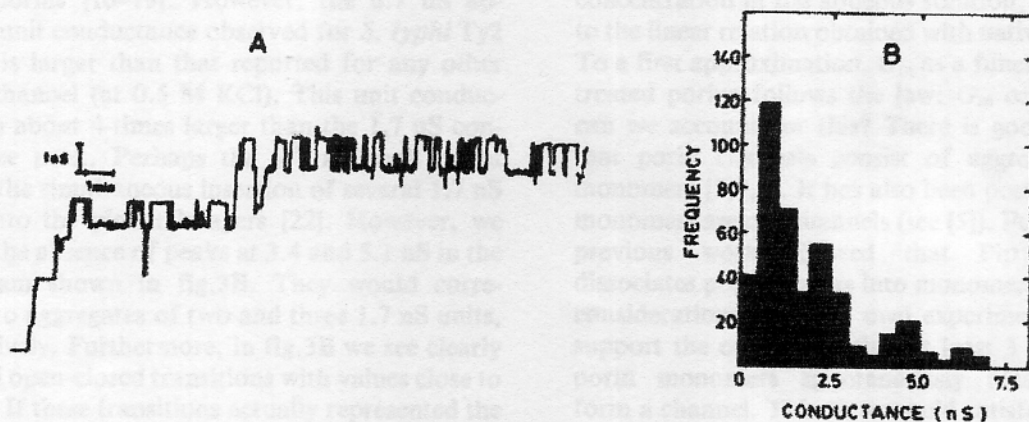


Fig.4. Discrete conductance fluctuations induced by Pip-modified porins in planar lipid bilayers. (A) Experimental record of discrete fluctuations in conductance. (B) Histogram showing jump-size distribution in bilayers treated with modified porins. The histogram was constructed from data similar to those in A and contains 500 discrete conductance jumps.

#### 4. DISCUSSION

Our results allow us to conclude that both native and Pip-treated porins from *S. typhi* Ty2 induce channel-mediated increases in the conductance of planar bilayers. Although these studies were obtained in bilayers made of oxidized cholesterol, similar observations have been made in bilayers composed of other lipids (P. Labarca, unpub-

lished). Thus, it seems reasonable to suggest that porin channels from this bacterium might also be incorporated in other lipid bilayers.

It was found that the magnitude of the conductance induced by native porins is a linear function of their concentration in the aqueous buffer bathing the bilayers. In this regard, porins from *S. typhi* Ty2 behave like those derived from other gram-negative bacteria [4-6]. These observations



Table 1

Average size of discrete conductance jump for native and Plp-treated porins

	Average conductance $\pm$ SE (nS)
Native porins	3.6 $\pm$ 0.4 ( $n=300$ )
Modified porins	2.1 $\pm$ 0.1 ( $n=400$ )

Values represent the average of 370 (native) and 500 (modified porins) discrete jumps

can be interpreted as evidence that porin channels are inserted into planar bilayers as preformed units.

The conductance histograms derived from bilayers treated with native porins reveal two major peaks at  $\sim 1.7$  and  $\sim 6.7$  nS. A broad distribution of conductance jumps has been reported for other porins [16-19]. However, the 6.7 nS apparent unit conductance observed for *S. typhi* Ty2 porins is larger than that reported for any other porin channel (at 0.5 M KCl). This unit conductance is about 4-times larger than the 1.7 nS conductance peak. Perhaps the 6.7 nS peak might reflect the simultaneous insertion of several 1.7 nS units into the planar bilayers [22]. However, we notice the absence of peaks at 3.4 and 5.1 nS in the histogram shown in fig.3B. They would correspond to aggregates of two and three 1.7 nS units, respectively. Furthermore, in fig.3B we see clearly defined open-closed transitions with values close to 6.7 nS. If these transitions actually represented the presence of aggregates of several 1.7 nS units, one would have to accept that these units can somehow act in concert to activate and shut off almost simultaneously. We have also noticed in our experimental records the occurrence of substrates of conductance in the large 6.7 nS channels. In addition, we have evidence that other chemical modifications can selectively abolish the large 6.7 nS channels, without affecting those of 1.7 nS. For example, treatment of native porins with phenylglyoxal [9], yields conductance histograms which lack the 6.7 nS peak. (P. Labarca, unpublished). Thus, although we are not able to discard the hypothesis that the 6.7 nS conductance channel represents aggregates of smaller units [22], we tentatively wish to propose that the two peaks

of conductance, at 1.7 and 6.7 nS, seen in conductance histograms are due to the occurrence of two open states. In summary, our results suggest the presence of large permeability channels in the outer envelope of *S. typhi* Ty2.

Reductive alkylation of *S. typhi* Ty2 porins with Plp renders them soluble in aqueous solution. Such an observation led Calderón et al. [9] to conclude that this modification dissociates porin oligomers into monomers. The present results show that these modified porins are still able to form channels in planar bilayers, albeit with smaller induced discrete conductance jumps. Benz et al. [5] have recently demonstrated that chemical modification of *E. coli* porins can alter their single channel conductance. However, the most striking difference between Plp-treated and native porins concerns their different effects on  $G_m$ . As illustrated in section 3, the conductance of bilayers exposed to modified porins is a logarithmic function of their concentration in the aqueous solution, in contrast to the linear relation obtained with native material. To a first approximation,  $G_m$  as a function of Plp-treated porins follows the law:  $G_m \propto p^{2.8}$ . How can we account for this? There is good evidence that porin channels consist of aggregates of 3 monomers [5,7,8]. It has also been postulated that monomers are not channels (see [5]). Furthermore, previous work showed that Plp treatment dissociates porin trimers into monomers [9]. These considerations plus our own experimental results support the conclusion that at least 3 Plp-treated porin monomers spontaneously reassociate to form a channel. This view would satisfactorily account for the power dependence of  $G_m$  on the concentration of modified porin. A power dependence of bilayer conductance on channel-former antibiotic concentration has been interpreted along similar 'aggregational' models [23]. Finally, such a conclusion would agree with the view that porin monomers are not channels [5,7,8].

Since reductive alkylation of proteins with Plp is specific for lysine residues [20], our results would indicate that an as yet undetermined number of lysine residues, probably located near the channel surface, are crucial in stabilizing them. The presence of several lysine residues near the surface of *E. coli* porins has been documented by Schindler and Rosenbush [21]. Further studies will define the number of Plp molecules bound per

porin monomer. Moreover, studies underway will allow us to establish whether this and other modifications alter the ion-conduction properties of *S. typhi* Ty2 porin channels.

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