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The major *Thiobacillus ferrooxidans* outer membrane protein forms low conductance ion channels in planar lipid bilayers

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A protein isolated and purified from the outer membrane of the acidophilic, chemolithotrophic bacterium, *Thiobacillus ferrooxidans* with an oligomeric molecular weight of 90 000 Da (p90) was incorporated into phosphatidylethanolamine planar lipid bilayers. The protein formed slightly anionic channels in KCl solutions, with a conductance of 25 pS in 100 mM KCl. The current–voltage relationship was linear between ±60 mV, and the conductance was a saturating function of the salt concentration. These channels fluctuated from a single open to closed state at low potentials, but present flickering activity at higher potentials.

Ion channel; Porin; Lipid bilayer; Autotrophic bacteria; *Thiobacillus ferrooxidans*

1. INTRODUCTION

The outer membrane of Gram-negative bacteria acts as a molecular sieve that allows the passage of ions and small hydrophilic organic molecules. This property is due to the presence of a major group of proteins, the porins, that form large diffusion pores [1–4]. Porins have been well characterized in *Escherichia coli* and *Salmonella typhimurium*. Their primary and secondary structures are known, especially for general diffusion porins, OmpC and OmpF [4–8]. Furthermore, they have been extensively biochemically and electrophysiologically characterized [1,9]. Reconstituted into planar lipid bilayers they form high conductance aqueous pores which open for seconds, have low ionic selectivity and voltage-dependent gating in most studies [10–13].

While porins have been the object of considerable studies, no information about the properties of the outer membrane proteins of autotrophic bacteria is available. Some of these prokaryotes have gained great interest due to its use in mining since they are able to produce leaching of mineral. Recently, Rodríguez et al. [14] have reported the purification method and physicochemical characterization of lipopolysaccharides and some proteins of the external membrane of the acidophilic, chemolithotrophic bacterium *Thiobacillus ferrooxidans*. Among them, its major outer membrane protein (p90) presents similarities to OmpC and OmpF porins of *E. coli* and *S. typhymurium*: its monomer has an apparent molecular weight of 40 000 and its association in sodium dodecyl sulfate resistant 90 000 Da oligomers with similar porin-like electrophoretic mobility.

In this communication, we report the reconstitution into planar lipid bilayers of p90 protein isolated and purified from the external membrane of *Thiobacillus ferrooxidans*.

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2. MATERIALS AND METHODS

2.1. *Isolation and purification of p90 outer membrane protein*

The p90 protein was purified according to the method described by Rodriguez et al. [14]. Membranes were prepared by passing a cell suspension in 10 mM Tris-Cl, pH 7.8, three times through a French press at 19 000 psi. After centrifugation at 12 000 × *g*, to eliminate undisrupted cells, the supernatant containing inner and outer membranes, was spun at 100 000 × *g* for 60 min. Total membrane fraction was then solubilized in a 10 mM Tris-Cl, 5 mM EDTA, 0.5% sodium *N*-laurylsarcosine (Sarkosyl) buffer, pH 7.8. The detergent-insoluble outer membrane fraction was then recovered by centrifugation at 100 000 × *g* for 45 min.

Isolation of p90, the major outer membrane protein was carried out by resuspending the outer membrane pellet in 1% SDS, followed by dialysis against water. Dialyzate was treated with trypsin (0.1 mg/ml) for 2 h at 37°C. Trypsin resistant fraction was further purified by gel filtration on a Sephacryl S-200 column. The purified polypeptide solubilized at room temperature in Laemmli's buffer [15], migrated as a single 90 kDa band in SDS-PAGE. On the other hand, if solubilization was done at 100°C, a single 40 kDa band in SDS-PAGE was obtained. This temperature-dependent electrophoretic behavior, is characteristic of several bacterial porins [16].

2.2. *Planar bilayers and channel incorporation*

Bilayers were formed according to the method of Mueller et al. [17] in a 300 μm diameter hole, made in a Teflon film of 25 μm thick separating two aqueous solutions. The phospholipid solution was brain phosphatidylethanolamine in decane (12.5 mg/ml). Bilayer resistances were always >50 GΩ and had capacitances in the range of 150–300 pF. Planar bilayers were formed at different KCl concentra-

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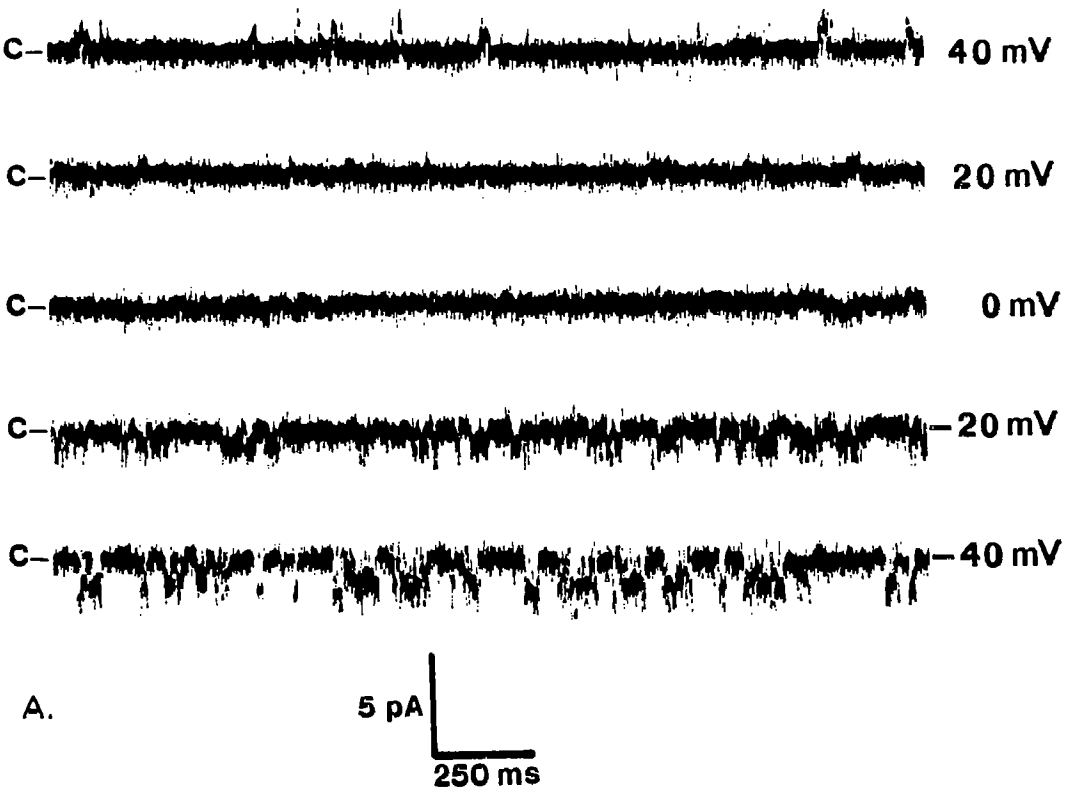

Fig. 1. (A) Recordings of unitary currents across the p90 channel in the presence of a 600 mM (*cis*)/300 mM (*trans*) KCl gradient, 5 mM symmetric citric acid/KOH, (pH 3.0) at the indicated voltages. C = indicates closed state. Records were filtered at 1 kHz.

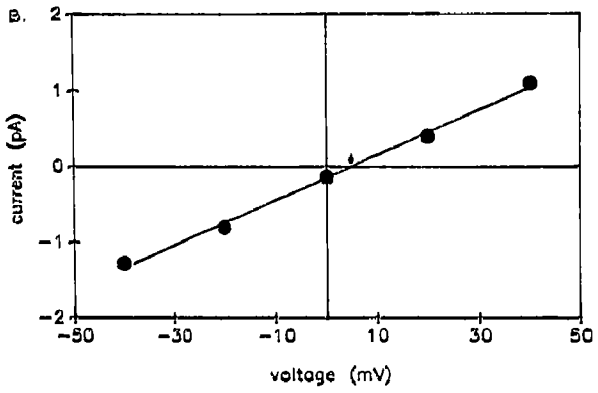

Fig. 1. (B) Current–voltage relationships of p90 channel under the conditions described in Fig. 1.

tions between 100 and 600 mM. All solutions used were buffered at pH 3.0 with 5 mM citric acid/KOH, in order to mimic that of the living environment of these bacteria. KCl gradients were formed by adding 3 M aliquots to the *cis* side.

Channel insertion was achieved by the addition of aliquots of protein stock solution (1 mg/ml) containing 0.1% SDS, to one of the compartments of the chamber to a final concentration of 3 μg/ml. Current was measured with a two-electrode voltage clamp [18]. One chamber compartment (*cis*), was connected to a voltage pulse generator and the opposite (*trans*) to a current–voltage converter, through Ag/AgCl electrodes. Electrodes were connected to the solution via agar bridges made in 1 M KCl, and the *trans* side was virtual ground. The current was low-pass filtered and stored in tape for further analysis.

3. RESULTS

Channel incorporation was observed as the sudden appearance of rapid discrete current fluctuations when a constant voltage was applied across the bilayer. The ionic selectivity of p90 channel was studied recording the unitary currents in a KCl concentration gradient, at different voltages (Fig. 1A). The current–voltage (*I/V*) curve of this channel between −50 and 50 mV is shown in Fig. 1B. The relationship is linear and the reversal potential is 6 mV. A permeability ratio, P_{Cl}/P_K of 2.1 was obtained using the Goldman-Hodgkin-Katz equation [19].

The conductance of the channel in symmetrical KCl solutions was determined at four concentrations between 100 and 600 mM to study the conductance–concentration relationship. Fig. 2 shows single channel recordings of the p90 channel in symmetric 600 mM KCl between −60 and 60 mV. It can be observed that at low potentials the channel presents a single open state but at higher potentials the records become noisier appearing short-lived openings (flickering). Fig. 3 shows the *I/V* relationships for the p90 channel at three different KCl concentrations. From the slopes of these curves the plot of conductance vs KCl concentration was built. Channel conductance was 25 pS at 100 mM KCl and it is a saturable function of salt concentration, in the range studied (Fig. 4). This function suggests the

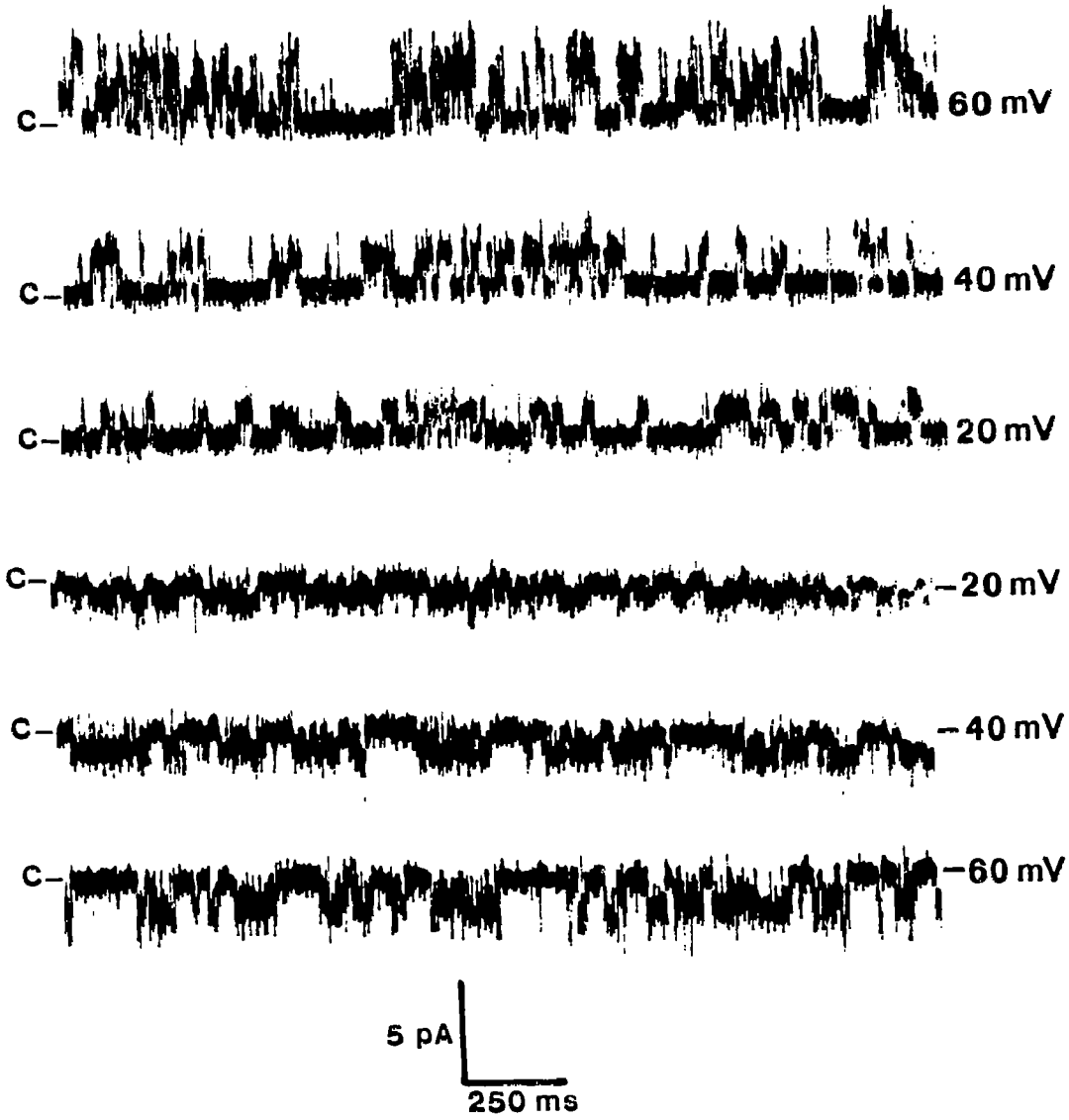

Fig. 2. Recordings of unitary currents across the p90 channel in symmetric 600 mM KCl, 5 mM citric acid/KOH (pH 3.0) at 6 voltages.

presence of ionic or saturable binding sites inside the channel.

When KCl was replaced by K_2SO_4 in the saline the channel became cationic. Fig. 5A shows a single channel recording of a p90 channel in a 330/165 K_2SO_4 gradient, at three different voltages. The *I/V* curve is linear (Fig. 5B) and a zero-current potential of −17 mV was obtained. The same value was predicted by the Nernst equation, indicating that the channel became strictly K^+ selective, excluding SO_4^{2-}.

4. DISCUSSION

The p90 oligomeric protein from the *Thiobacillus ferrooxidans* outer membrane, incorporated into planar lipid bilayers induced the appearance of low conduct-

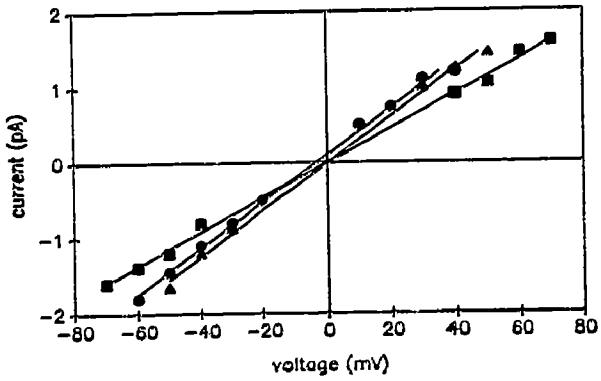

Fig. 3. Current–voltage relationships of p90 channel in symmetric 100, 300 and 600 mM KCl, 5 mM citric acid/KOH, pH 3.0.

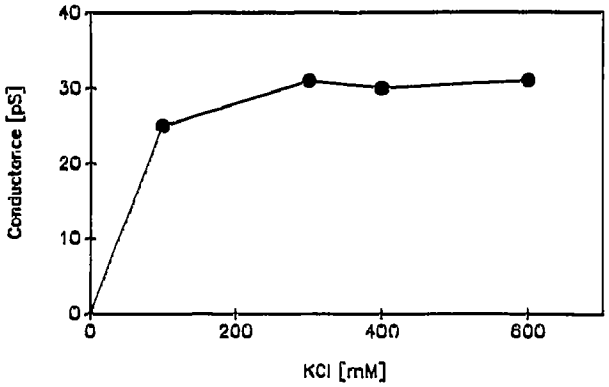

Fig. 4. Conductance of p90 channel as function of KCl concentration under the conditions described in Fig. 3.

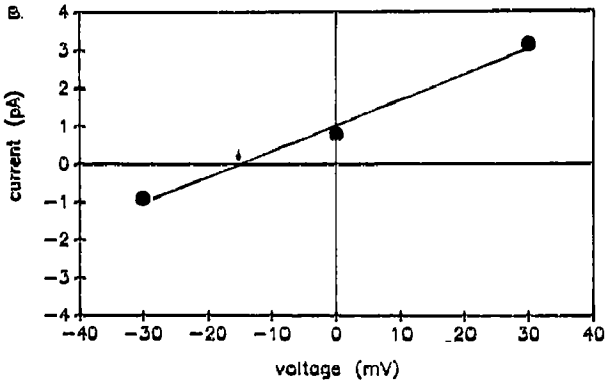

Fig. 5. (B) Current–voltage relationship of p90 channel under the conditions described in Fig. 5A.

ance ionic channels in KCl solutions. These channels present two conductance states at low voltages and a flickering activity at higher potentials. Most of the studies of porin characterization in planar lipid membranes have shown that these proteins form high conductance pores with values of about 300 pS in symmetrical 150 mM KCl [20,21]. However, smaller values are now being reported in the literature. Delcour et al. [22] found that the conductance of wild-type OmpC porin trimer from *E. coli*, was about 162 pS in 150 mM KCl, with a unit conductance of 56 pS. On the other hand, Maier et al. [23] reported that Tsx protein from the outer membrane of *E. coli* formed a channel with a conductance of 10 pS in 1 M KCl. The conductance values found in this study represent one of the smallest reported for a bacterial outer membrane protein channel.

Regarding the selectivity, p90 channel does not discriminate well between K^+ and Cl^-, similar to general diffusion porins. However, in K_2SO_4, the channel becomes cation selective. This finding suggests that the conduction pathway is narrow enough to prevent the passage of sulfate ions.

Although we have not reconstituted p90 protein in liposomes in order to study non-electrolyte permeability by the swelling technique, preliminary osmotic experiments with intact cells indicate that neither stachyose nor sucrose permeate through the outer membrane of *Thiobacillus ferrooxidans*.

In summary, our results indicate that p90 protein forms small pores that would allow the passage of small ions and molecules. This finding is consistent with the autotrophic nature of these bacteria.

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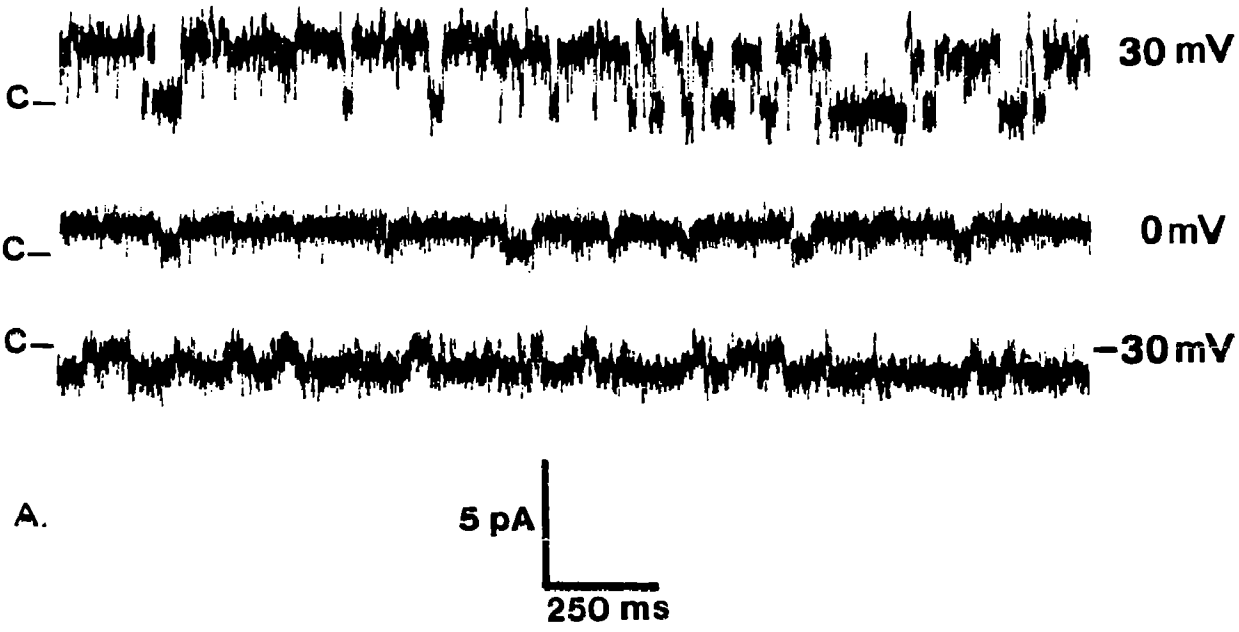

Fig. 5. (A) Single p90 channel recordings in a 330 mM (*cis*)/165 mM (*trans*) K_2SO_4 gradient, 5 mM citric acid/KOH, pH 3.0.

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