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Outer membrane porin protein of *Campylobacter jejuni*

(*Campylobacter jejuni*; outer membrane protein; porin)

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1. SUMMARY

Protein e, a 43-kDa protein from the outer membrane of *Campylobacter jejuni* UA580, was purified and reconstituted into lipid bilayer membranes. It was shown to form small channels with a single channel conductance of 8.82 nS in 1M KCl. Zero current potential measurements demonstrated that the channel was approx. 10-fold selective for K⁺ over Cl⁻ ions. A porin with a similar single channel conductance was observed in fractions from the outer membrane of *Campylobacter fetus* UA60.

2. INTRODUCTION

C. jejuni, a Gram-negative, microaerophilic bacterium, has recently emerged as one of the primary pathogens involved in diarrhea and acute enteritis in North American adults.

The outer membranes of a variety of *C. jejuni* strains were isolated by Logan and Trust [1]. They demonstrated that each of the strains examined contained a major outer membrane polypeptide species, protein e, with a M_r approx. 45 000. Protein e is a trans-membrane protein, as judged by

its interaction with the peptidoglycan and by its ability to be surface-iodinated using the lactoperoxidase system [1]. Furthermore, protein e from different *C. jejuni* strains is immunologically cross-reactive [2]. Outer membrane proteins of similar molecular weight from *C. fetus* and *Campylobacter coli* strains also reacted with anti-serum to *C. jejuni* formalinized cells. These data suggest that protein e is a common antigen in *Campylobacter* species. Logan and Trust [1] hypothesized that protein e was a porin protein and may form hydrophilic channels across the outer membrane. In this paper we have purified protein e from *C. jejuni* strain UA580 (NCTC11168) and demonstrated that it will reconstitute small ion-permeable channels in lipid bilayer membranes.

3. MATERIALS AND METHODS

Strains *C. jejuni* UA580 (NCTC11168) and *C. fetus* ssp. *fetus* UA60 (obtained from D. Taylor, Department of Medical Microbiology, University of Alberta) were grown in 600-ml volumes of Mueller Hinton broth (Difco Laboratories, Detroit, MI) contained in 3-l Fernbach flasks. Cultures were incubated statically at 37°C in an atmosphere of air enriched with 8% CO₂, at 85%

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humidity. *C. jejuni* or *C. fetus* cells were harvested after 24 or 48 h incubation, respectively, yielding 0.4 g wet weight of cells per 600 ml culture. The cells were active, motile and present as spiral forms, with no sign of the aging coccoid forms. The cells were harvested by centrifugation, washed once with 8 mM Tris-HCl buffer, pH 7.8, and suspended in 8 mM Tris-HCl, pH 7.8, containing 50 μ g/ml DNase and RNase. The ice-cold suspension was passed once through a French pressure cell at maximum pressure and then treated with 0.1 μ /ml (final concentration) lysozyme at 20°C for 30 min and 0°C for 60 min. Cell debris was removed by centrifugation at 17400 \times g for 30 min. The supernatant fluid was centrifuged at 177000 \times g for 30 min at 4°C to pellet the cell envelope. This pellet was resuspended in 20 mM Tris-HCl, pH 7.4, and the cytoplasmic membrane material was solubilized with 2% (v/v) Triton X-100 in a sonic water bath as described previously [3]. Outer membrane material was sedimented by centrifugation at 177000 \times g for 60 min and was solubilized in 2% Triton X-100/10 mM EDTA in 20 mM Tris-HCl, pH 7.4 [3]. Triton/EDTA-insoluble material was removed by centrifugation at 177000 \times g for 60 min at 4°C. Sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE) was conducted as described previously [4], using a 10% separation gel and a running buffer containing 0.1% SDS in 25 mM Tris/0.19 M glycine, pH 8.45 [4].

The methods used for black lipid bilayer experiments have been described previously in detail [5,6]. The apparatus consisted of a Teflon chamber with 2 compartments connected by a small hole (0.1–2 mm²). A membrane was formed across the hole by painting on a solution of 1–2% (w/v) oxidized cholesterol in *n*-decane. Bilayer formation was indicated by the membrane turning optically black to incident light. Conductance through the pores was measured after application of a given voltage, using a pair of Ag–AgCl electrodes inserted into the aqueous solutions on either side of the membrane. The current through the pores was boosted by a preamplifier, monitored by a storage oscilloscope, and recorded on a strip chart recorder. Zero-current potential measurements were performed as described previously [5,6].

4. RESULTS AND DISCUSSION

The triton/EDTA soluble fraction of the *C. jejuni* envelope demonstrated one major heat-modifiable protein which had an apparent M_r of 43 000 after solubilization in SDS at 80°C. This preparation therefore, contained substantially purified protein e. The protein demonstrated no oligomer form on SDS-polyacrylamide gels after solubilization at low temperature (Fig. 1) in contrast to many porins from other bacteria [7]. Instead, after solubilization at 37°C in SDS, the protein ran

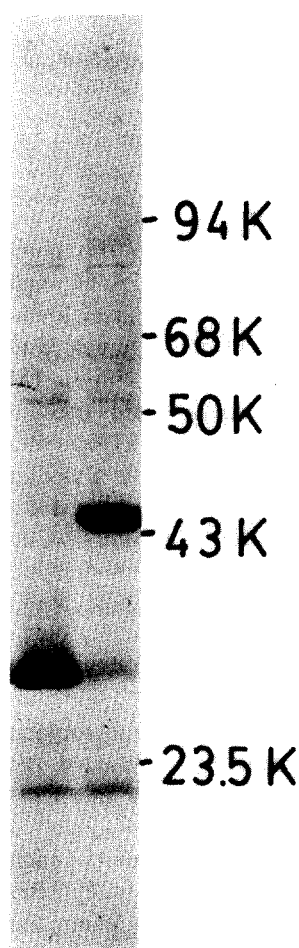


Fig. 1. SDS-polyacrylamide gel electrophoretogram of *C. jejuni* purified protein e. Samples were solubilized at 37°C (lane A), 55°C (lane B) or 80°C (lane C) prior to electrophoresis. The running positions of molecular weight standards are shown on the right-hand side.

with an apparent M_r of 31 000 (Fig. 1, lane A) and shifted to an apparent molecular weight of 43 000 after solubilization in SDS at 55°C (Fig. 1, lane B) or higher. Similarly treated outer membrane preparations, prepared by Sarkosyl extraction, also showed no heat-labile oligomer on SDS-polyacrylamide gels (data not shown). This partially contradicted the data of Logan and Trust [1] who suggested that protein e ran partly as an 'oligomeric complex' of > 92 000 and partly as a 33 000 band after solubilization at 37°C. However, it should be noted that no evidence for the identity of these various bands was presented.

Addition of moderate amounts of protein e (1 ng/ml final concentration) to one or both of the solutions bathing a lipid bilayer membrane resulted in a 3 orders of magnitude increase in conductance over a period of 30 min. At this time the membrane current was a linear function of applied voltage suggesting that the pores responsible for the observed current were not voltage-regulated or gated. Heating the protein e preparation to 100°C for 5 min in SDS destroyed its channel-forming activity.

Addition of smaller amounts of protein e (0.2 ng/ml final concentration) to the aqueous phase (1 M KCl) bathing the lipid bilayer membrane

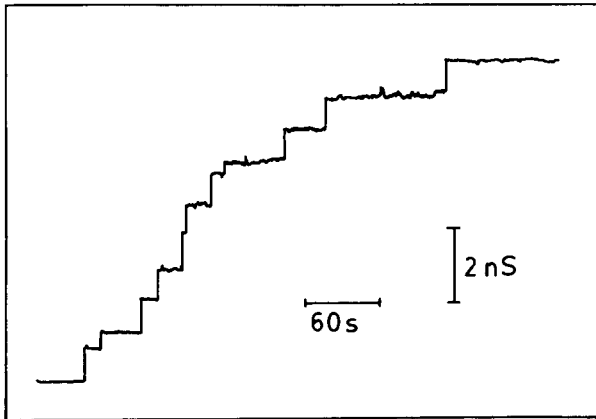


Fig. 2. Stepwise increases in membrane conductance after the addition of the *C. jejuni* protein e preparation to a final concentration of 0.2 ng/ml to the 1 M KCl solution bathing a lipid bilayer membrane. The trace starts on the left-hand side, at which time protein e was added. Prior to this, the membrane current was 10^{-11} A.

allowed resolution of the conductance increase into individual steps (Fig. 2). By analogy with other lipid bilayer experiments, these conductance increments were presumed to involve the incorporation of single channel-forming units of protein e into the lipid bilayer. As with other porins [5], the conductance increments were not uniform but distributed around a mean. The average single channel conductance in 1 M KCl was 0.82 ± 0.23 nS (Table 1), a value smaller than that for most other constitutive major outer membrane porin proteins [6]. At lower concentrations of KCl, smaller single channel conductances were observed, although

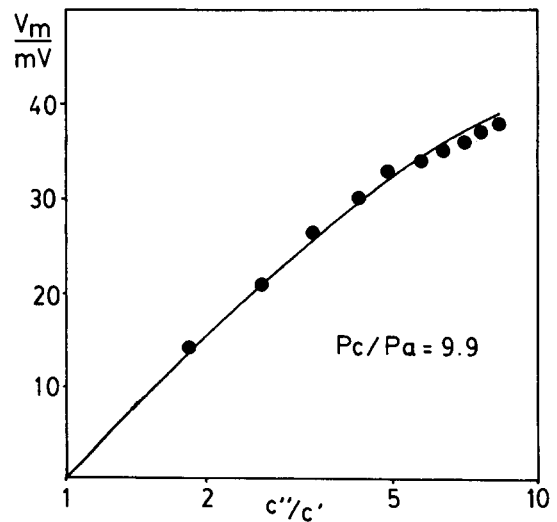


Fig. 3. Zero current potential measurements using *C. jejuni* protein e. Approx. 100–1000 protein e pores were allowed to incorporate into a lipid bilayer membrane in the presence of 80 mM KCl. At this time the voltage was turned off and a concentration gradient established across the membrane by the addition of equal aliquots of 3 M KCl to the concentrated side (c'') of the membrane and of 50 mM KCl to the dilute side (c') of the membrane. The resulting concentration gradient served as a driving force for ion movement which occurred according to the ion selectivity of the channel. The preferential movement through the protein e channels, in this case, of cations created a positive potential which opposed the movement of ions. The measured potential (V_m) at the time when ions ceased to flow was the zero current membrane potential and increased for increasing concentration gradients. A typical experiment is shown. The line is drawn by application of the Goldman-Hodgkin-Katz equation [6] to the given concentration gradients assuming a ratio of $K^+ : Cl^-$ permeabilities through protein e channels of 9.9:1.

Table 1

Average single channel conductances observed in the presence of *C. jejuni* protein e in a variety of different salt solutions. The average single channel conductance Λ was obtained for each salt as the average of a large number of single channel events similar to those shown in Fig. 2. The bulk conductance σ of the given salt solution is the conductance of the salt solution in the absence of a membrane.

Salt	Concentration (mM)	Single channel conductance Λ (nS)	Bulk conductance σ (μ S/cm)	Λ/σ	Number of events measured
KCl	1000	0.82	110	0.75	213
	500	0.62	56	1.11	100
	100	0.20	13	1.54	121
	30	0.14	3.5	2.50	144
NaCl	1000	0.57	84	0.68	144
LiCl	1000	0.40	71	0.56	176
MgCl ₂	1000	0.21	128	0.16	125
Tris ⁺ Cl ⁻	500	0.05	30	0.17	130
Na ⁺ Hepes ⁻	500	0.20	18	1.11	116

there was no precise linear relationship between the average single channel conductance and the bulk conductivity for different concentrations of KCl (Table 1).

Similar measurements of the single channel conductance of protein e were made in the presence of other salts (Table 1). The data suggested that the channel was cation selective since the use of salts with large cations such as Mg²⁺ and (Tris)⁺ resulted in a relative decrease in conductance, whereas the use of (Hepes)⁻ instead of Cl⁻ had far less effect on the average single channel conductance. To confirm the selectivity of the channel, zero current potential measurements were performed and fitted to the Goldman-Hodgkin-Katz equation (Fig. 3). The data suggested that the ratio of permeabilities of cations (P_c) and anions (P_a), $P_c:P_a$ was $9.9 \pm 1.9:1$ for KCl, thus confirming the cation selectivity of the channel.

Black lipid bilayer experiments with the equivalent *C. fetus* protein preparation clearly revealed 2 distinct single channel conductances with means of 0.82 ± 0.16 nS and 1.62 ± 0.30 nS (460 measured events). This preparation contained 2 major outer membrane proteins of 45 and 48 kDa, which we were unable to successfully separate. Our working hypothesis is that both the 45- and 48-kDa outer membrane proteins of *C. fetus* are porins. Consistent with this, Logan and Trust [1] showed that the 48-kDa band apparently cross-reacts anti-

genically with *C. jejuni* protein e, and we observed channels with identical mean conductances from both species.

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