THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 42, Issue of October 15, pp. 29607–29612, 1999

Printed in U.S.A.

Porin Is Present in the Plasma Membrane Where It Is Concentrated in Caveolae and Caveolae-related Domains*

(Received for publication, June 16, 1999)

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Mitochondrial porin, or voltage-dependent anion channel, is a pore-forming protein first discovered in the outer mitochondrial membrane. Later investigations have provided indications for its presence also in other cellular membranes, including the plasma membrane, and in caveolae. This extra-mitochondrial localization is debated and no clear-cut conclusion has been reached up to now. In this work, we used biochemical and electrophysiological techniques to detect and characterize porin within isolated caveolae and caveolae-like domains (low density Triton-insoluble fractions). A new procedure was used to isolate porin from plasma membrane. The outer surface of cultured CEM cells was biotinylated by an impermeable reagent. Low density Triton-insoluble fractions were prepared from the labeled cells and used as starting material to purify a biotinylated protein with the same electrophoretic mobility and immunoreactivity of mitochondrial porin. In planar bilayers, the porin from these sources formed slightly anion-selective pores with properties indistinguishable from those of mitochondrial porin. This work thus provides a strong indication of the presence of porin in the plasma membrane, and specifically in caveolae and caveolae-like domains.

Caveolae are plasma membrane organelles, characterized by a low solubility in Triton X-100, where specific lipid and protein components are subcompartmentalized. They have been proposed to function in transcytosis, potocytosis (involving GPI-linked receptors and (an) unknown anion carrier(s) or pore(s)), and signal transduction (1–3). Caveolins, a family of integral membrane proteins, are the structural proteins of caveolae; they form a "scaffold" on which a variety of signaling molecules are organized in complexes (3). In cells not expressing caveolin, analogous microdomains are formed by association of sphingolipids and cholesterol. They have been referred to as DIGs (detergent-insoluble glycolipid-enriched complexes) (4) or, as in this study, as low density Triton-insoluble complexes (LDTIs)[1] (5). We have previously established a reliable procedure to isolate caveolae and LDTI from tissues or cultured cells (6), which allowed us to compile a partial catalogue of caveolar proteins (7). In the mouse lung preparation, >98% of generic plasma membrane protein markers and >99.6% of organelle (Golgi, lysosomes, endoplasmic reticulum) markers were excluded, whereas >85% of caveolin was retained. Porin was tentatively identified as a caveolar component by microsequencing an SDS-PAGE band (7).

Porin, or VDAC, is an integral membrane protein of about 35 kDa forming a pore permeable to solutes of molecular mass <2 kDa in the outer mitochondrial membrane (8, 9). Its most relevant biophysical properties upon reconstitution in planar bilayers include a maximal conductance of about 4.5 nanoSiemens in 1 M KCl, a symmetrical voltage dependence leading to closures at potentials higher than about ±20 mV, the presence of many subconductance levels, a slight selectivity for anions in the fully open state, and a variable kinetic behavior with both slow and fast gating modes (10). The lack of porin in muscle biopsies has been associated with an inherited fatal encephalomyopathy (11). In higher eukaryotes, the existence of multiple genes (12) and transcripts (13) has recently been established, but little is known about the role(s) and subcellular compartmentalization of porin isoforms and about a possible modulation of the pore, which was believed until recently (10), to be unable to close completely. In 1989, Thinnes and co-workers (14, 15) reported the presence of the porin isoform 31-HL (or HVDAC1) in the plasma membrane of human B lymphocytes. The presence of VDAC in the plasma membrane of at least some cell types has since been supported (16, 17), but the finding has been disputed (18) and the issue remains undecided. The aim of this work was thus to establish whether porin is present in the plasma membrane, and specifically in caveolae or caveolae-related domains, of hematopoietic and brain cells. This question is of high biological relevance, because the main recognized task of porin is to create a large, rather unspecific, channel, apparently incompatible with plasma membrane function.

EXPERIMENTAL PROCEDURES

Isolation of Caveolae, LDTI, and Mitochondria—Caveolae-rich fractions were prepared from organs (rat heart, bovine brain and dog lung) using a modification of a procedure that has previously been developed for lung (7). 0.4–0.5 g (wet weight) organ tissue was snap-frozen and

* This work was supported in part by Telethon-Italy Grants A44 and A59 (to M. Z.), Grant 711 (to V. D. P.), and a fellowship (to G. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‖ Supported by a post-doctoral fellowship from the University of Padova.

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[1] The abbreviations used are: LDTI, low density Triton-insoluble fraction; NHS-SS-biotin, sulfosuccinimidyl-2-[biotin-amido]-ethyl-1,3-dithiopropionate; VDAC, voltage-dependent anion channel; Mes, 4-morpholineethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; Abs, antibodies; PKCθ, protein kinase C θ; UTR, untranslated region.

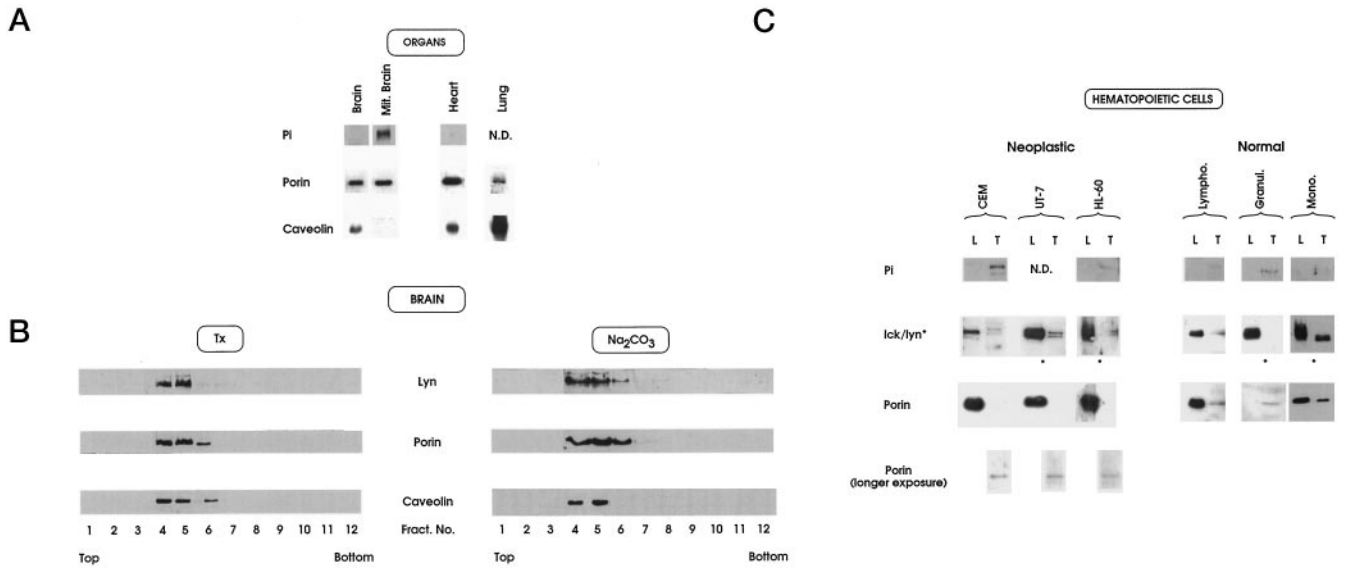

FIG. 1. **Porin detection in caveolae or LDTI from various tissues and cell lines.** *A,* 20 μg of protein from bovine brain, rat heart, and dog lung caveolae were analyzed by Western blotting for porin, caveolin, and the mitochondrial phosphate carrier (P_i). Brain, heart and lung caveolae were incubated with polyclonal anti-caveolin, monoclonal anti-caveolin 3, and monoclonal anti-caveolin 1, respectively. Porin and P_i were assayed with polyclonal Abs. *Brain*, *heart*, *lung*, caveolae from the respective organs; *mit. brain*, brain mitochondria. *B,* bovine brain was treated with 1% Triton X-100 or 0.5 M Na_2CO_3 and subjected to a discontinuous 5–40% sucrose density gradient centrifugation. Twelve fractions were collected. Fractions containing 25 μg of protein were analyzed by Western blotting with polyclonal Abs against lyn (a Src protein), mitochondrial porin, or caveolin. *C,* LDTI (*L*) were obtained by the Triton procedure from human hematopoietic neoplastic (CEM, UT-7, HL-60) and normal (lymphocytes, granulocytes, monocytes) cells. 20-μg fractions from LDTI (*L*) and total cell lysate (*T*) were separated, blotted, and immunostained with polyclonal Abs anti-P_i, anti-porin and anti-lyn or lck.

ground. Each sample was resuspended in 3 ml of 1% Triton X-100 MBS (25 mM Mes, pH 6.5, 0.15 M NaCl) or in 0.5 M Na_2CO_3, pH 11 (detergent-free procedure, see Ref. 19), and homogenized. A quarter of the tissue homogenate was adjusted to 40% sucrose, placed at the bottom of an ultracentrifuge tube and overlaid with 30 and 5% sucrose buffered with MBS or Na_2CO_3, to form a three-step discontinuous gradient. After centrifugation (14–17 h) in a SW60 rotor at 45,000 rpm, 4 °C, a sharp band (LDTI) was collected between the 5 and 30% sucrose layers. Alternatively, 12 fractions were collected from the top of the centrifuge tube and analyzed. Normal hematopoietic cells (granulocytes, lymphocytes, monocytes) from human peripheral blood and neoplastic counterparts (CEM, UT-7, HL-60) were fractionated, and LDTI was obtained by similar procedures (5). Mitochondria were isolated by standard procedures.

Porin Purification from Caveolae and LDTI—1 ml of caveolae or LDTI in MBS (about 10 mg of protein) was solubilized for 30 min at 4 °C with 1 ml of 3% Triton X-100, 10 mM Tris-HCl, pH 7.0, 1 mM EDTA. The suspension was then spun down and the supernatant (ET1) recovered; the pellet was again solubilized with 1 ml of 10% Triton X-100, 10 mM Tris-HCl, pH 7.0, 1 mM EDTA. Solubilization and centrifugation yielded supernatant ET2. Fractions ET1 and ET2 were loaded onto separate hydroxylapatite/celite (Bio-Rad, Serva) 2:1 dry columns (0.3 g) (20). Elution was performed with solubilization medium. Three fractions were collected, corresponding to the unretarded material and to the following 0.6-ml portions.

Purification of Biotinylated Plasma Membrane Porin—CEM cells (an established T lymphoid-like cell line) were washed three times with phosphate-buffered saline without calcium and magnesium and incubated with NHS-SS-Biotin (Pierce) (0.5 mg/ml; 30 min, 4 °C). The cells were then processed to obtain the LDTI fraction. The porin purification procedure described above was performed on this material. The fraction containing the HTP/celite pass-through was incubated for 4 h with an equal volume of swollen immobilized streptavidin-agarose (Pierce), pre-equilibrated with solubilization medium. After two washing steps, the agarose was incubated with 0.1 M dithioerythritol for 30 min. After centrifugation, the supernatant was analyzed by SDS-PAGE.

Western Blotting Analysis and Immunoprecipitation Experiments—Total cell lysate was obtained by lysis (30 min at 4 °C) with 0.5 ml of 150 mM NaCl, 10 mM Tris, pH 7.4, 1% Nonidet P-40, 10% glycerol plus protease inhibitors (aprotinin, 1 μg/ml; pepstatin A, 1 μg/ml; leupeptin, 1 μg/ml; phenylmethylsulfonyl fluoride, 100 μg/ml), followed by centrifugation. Proteins were resolved by SDS-PAGE and blotted to nitrocellulose. The primary antibodies used are specified where appropriate; they were from commercial sources unless specified. Anti-porin Abs were used at a dilution of 1:1,000, and anti-P_i Abs at 1:40,000. Blots were developed (secondary antibody-horseradish peroxidase, Life Technologies, Inc.) using a chemiluminescence kit. The immunoprecipitation was performed by mixing 20 μg of LDTI domains obtained from NHS-SS-Biotin-treated CEM cells, precleared with 30 μl of a 50% protein A/G-agarose suspension (Pierce), and 1 μl of nonimmune serum in 0.5 ml of lysis buffer for 1 h at 4 °C. 0.5 μg/ml anti-CD4 or 1 μg/ml anti-PKCθ Abs were then added to the sample, and kept overnight at 4 °C, followed by incubation with pre-washed beads (40 μl), 1 h at 4 °C. The beads were washed 4 times, resuspended in 30 μl of sample buffer, boiled, and centrifuged. The supernatant was run on SDS-PAGE and immunodetected with anti-CD4 or with anti-PKCθ.

Planar Lipid Bilayer Experiments—Experiments were conducted as has previously been described (21). Purified asolectin (Sigma), phosphatidylethanolamine, or diphytanoylphosphatidylcholine (Avanti) membranes were prepared by painting a decane solution across a hole in a Teflon film separating cis and trans chambers. The experimental medium was 0.1, 0.5, or 1 M KCl plus 0.1 mM $CaCl_2$, 20 mM Hepes, pH 7.2. Voltages of the cis side (where porin was added) are reported. Current (cations) flowing from the cis to the trans compartment is considered positive and plotted upward.

RESULTS

We tested caveolae and mitochondria isolated from different organs (bovine brain, rat heart, and dog lung) for the presence of caveolin 1 or 3, of a mitochondrial marker, namely the phosphate carrier (P_i), and of porin. The anti-porin antibodies were raised either against the whole purified porin from bovine heart mitochondria or against a synthetic peptide mimicking the N-terminal end of HVDAC1 (22). These antibodies did not show any cross-reactivity with sequences from HVDAC2 (23) nor with any cellular protein other than HVDAC1.[2] Fig. 1*A* shows the Western blots obtained applying these antibodies to purified caveolae or mitochondria. Although caveolin was detected in caveolae and the phosphate carrier in mitochondria, porin was present in both fractions. The presence of caveolae in nervous tissue has been recently established (24, 25). Furthermore a procedure for the purification of caveolae using Na_2CO_3

[2] V. De Pinto, unpublished observations.

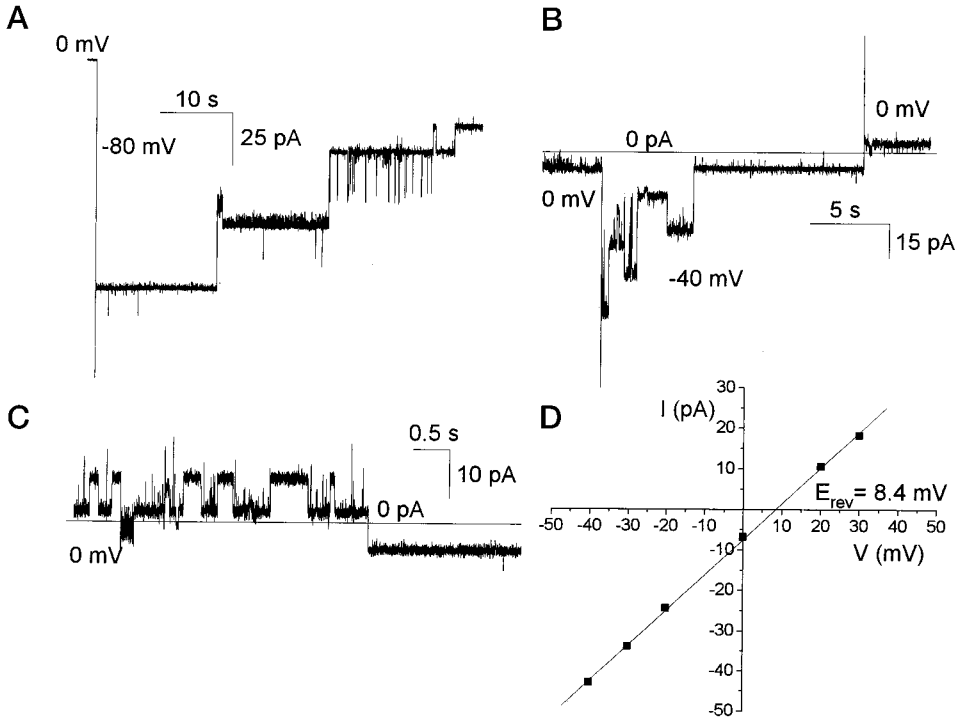

FIG. 2. **VDAC activity in caveolae from brain and LDTI from CEM cells.** *A,* a typical trace showing voltage-induced closures. Brain caveolae in an asolectin membrane, 1 M KCl. The capacitive spike on the *left* signals the transition from 0 to −80 mV. *B,* a trace illustrating voltage dependence and the adoption of both anion- and cation-selective states. Brain caveolae in an asolectin membrane. 390:100 (cis:trans) mM KCl. Anionic (cationic) selectivity is indicated by the flow of a negative (positive) current at zero potential. *C*, gating at zero potential. CEM LDTI; conditions as in *B. D,* I/V plot from an experiment with CEM LDTI in asolectin, 390:100 (cis:trans) mM KCl. Conductance: 0.88 nS; E_{rev}, 8.4 mV; P_{Cl}/P_K, 1.8.

and no detergent (19) was compared with the usual Triton isolation procedure. Protein from sucrose gradient fractionation were resolved by SDS-PAGE and stained in parallel with antibodies against porin, caveolin, and lyn (Fig. 1*B*). Lyn and lck are Src-family tyrosine kinases selectively recovered in LDTI from human leukemia cell lines and granulocytes (5). Porin distribution exactly matched those of caveolin and lyn also in the detergent-free procedure.

Several studies have pointed to the existence of porin in the cellular membrane of B and T lymphocytes. Thus, we assessed the presence of plasma membrane porin in hematopoietic cells, in which glycolipid-enriched domains (LDTI) likely represent the caveolae counterpart (3, 5, 27). Neoplastic (T-lymphoid (CEM), megakaryocytic (UT-7), promyelocytic (HL-60)) and normal (lymphocytes, granulocytes, monocytes) human hematopoietic cells were screened for porin expression (Fig. 1*C*). In every cell line the comparison between total cell protein extracts (T) and LDTI domains (L) indicated porin enrichment in the latter. The only exceptions were granulocytes (Fig. 1*C*) and red blood cells (not shown), in which porin was absent. This observation suggests that porin expression in the plasma membrane is regulated and possibly tissue- or cell-specific. The absence of porin in granulocyte LDTI further indicates that LDTI-associated porin in other cell types was not just a contaminant. Hematopoietic LDTI resident proteins, lck and lyn (5), were also detected. Our mitochondrial marker, the phosphate carrier (P_i), was absent from LDTI domains.

The functionality of porin in caveolae was assayed by incorporation of brain caveolae and CEM cell LDTI, prepared by the Triton method, into planar bilayers. VDAC-like channel activity (Fig. 2) was observed in 11 of 18 experiments with brain caveolae and in 22 of 27 experiments with CEM LDTI. These pores displayed single channel conductances matching the range of mitochondrial porin that were proportional to the salt concentration (at least up to 1 M KCl). They showed also voltage-induced closure at both positive and negative potentials (Fig. 2, *A* and *B*), both slow and fast kinetic modes, and both cation- and anion-selective conductance states (Fig. 2, *B-D*). These channel characteristics were in agreement with the known properties of VDAC. *Panel B*, taken from an experiment in 390:100 (cis:trans) mM KCl, illustrates a "memory effect". A channel in a high conductance, anion-selective state (negative current flowing) at zero applied potential, closed partially to a cation-selective substate upon application of −40 mV, and remained cation-selective upon returning to zero potential (positive current flowing). We routinely observed channel gating, including transitions between states of opposite selectivity, at 0 applied voltage (Fig. 2*C*), a behavior exhibited also by mitochondrial porin.[3]

We next isolated porin from bovine brain caveolae. The usual porin purification procedure (20) was first attempted on caveolae, but porin from this source turned out to be poorly soluble in 3% Triton X-100. We thus used a 10% Triton X-100 solution, which was more effective for porin solubilization. Elution from the HTP/celite column was performed with 3% Triton X-100. An SDS-PAGE of protein fractions obtained during the purification is shown in Fig. 3. The procedure yielded a single protein band of about 35 kDa (*lanes 3* and *4*), co-migrating with porin purified from bovine heart mitochondria (*lane Po*). This porin preparation exhibited a behavior indistinguishable from that of mitochondrial porin in planar lipid bilayer experiments (not shown).

In control experiments, we subjected mitochondria purified from bovine heart and rat liver to sucrose density centrifugation according to the protocol used for the isolation of caveolae. In these experiments, we observed that the mitochondrial porin could band between the 5 and 30% sucrose layers (data not shown). This result was indeed relevant, because porin has been demonstrated to be associated with sterol molecules both in *Neurospora crassa* (50) and in mammals (31). It posed the question of whether our caveolae preparations might be contaminated by mitochondrial proteins. We mentioned the absence of P_i carrier in LDTI; in addition, Western blots performed with Abs against four different rat liver mitochondrial outer membrane proteins did not show any partitioning in the

[3] G. Bàthori, unpublished observations.

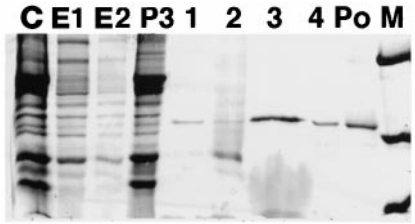

FIG. 3. **Porin purification from bovine brain caveolae.** A purification protocol consisting of two consecutive Triton X-100 solubilizations and HTP:celite chromatography was applied. The figure shows an SDS-PAGE Coomassie Blue staining (denaturing conditions). *C*, caveolae; *E1,* 3% Triton X-100 solubilized material; *E2,* 10% Triton X-100 solubilized material; *P3*, 10%Triton-unsolubilized material; *1*, first fraction eluted after the application of E1 to an HTP:celite dry column; *2*, second fraction, as in 1; *3*, first fraction eluted after the application of E2 to an HTP:celite dry column; *4*, second fraction, as in 3; *Po*, porin isolated from bovine heart mitochondria; *M*, molecular mass markers (from top): bovine serum albumin (68 kDa), carbonic anhydrase (31 kDa), and cytochrome C (12.5 kDa).

LTDI fraction in similar experiments (see Fig. 5 in Ref. 26). These results might mean that porin may exist in mitochondrial microdomains excluding most mitochondrial proteins but having the same density as caveolae or LTDI.

To rule out the possibility that the porin in caveolae might be just due to a contamination, we modified our purification procedure including a preliminary biotinylation step with an impermeable reagent: this was aimed at selecting proteins with domains exposed on the surface of the cell. We used NHS-SS-Biotin, a hydrophilic reporter containing an S-S bridge between the reactive moiety and biotin, to label CEM cells. To assess the impermeability of the cellular membrane to NHS-SS-Biotin, two assays were performed: (i) dye exclusion following biotinylation, to test cell integrity, and (ii) immunoprecipitation of plasma membrane marker proteins, CD4 and PKCθ, followed by streptavidin staining, to rule out the accessibility of the inner side of the plasma membrane to our reporter molecule. CD4 is a transmembrane protein localized in lymphocyte LDTI (5), containing hydrophilic domains exposed outside the cell. PKCθ is a plasma membrane protein, which does not show any hydrophilic moiety on the outer side of the membrane (27). PKCθ, CD4, and porin co-partitioned in the sucrose density fractions containing LDTI (Fig. 4*A*). After biotinylation, CEM cells were assayed for Trypan blue exclusion. 99% of biotinylated CEM cells were viable (not shown). They were processed to obtain LDTI. 20 μg of protein was immunoprecipitated with Abs anti-CD4 or anti-PKCθ, and the proteins were revealed by immunostaining with the same antibodies or with streptavidin (Fig. 4*B*). Only CD4 was detectable by streptavidin, indicating that only protein domains exposed to the outer side of the plasma membrane were biotinylated.

Biotinylated LTDI from CEM cells were subjected to the porin purification procedure described above. It resulted in a doublet of very close bands, as shown in Fig. 5*A*. These two bands represent modified (lower mobility band) and unmodified (higher mobility band) porin molecules, as established by comparative staining with anti-porin Ab and streptavidin-horseradish peroxidase conjugate (not shown). The reason for the partial porin unavailability to NHS-SS-biotin labeling is unclear. It might have been due to technical reasons (*i.e.* labeling conditions) or to the protein transmembrane disposition; it is well known that porin is deeply embedded in the phospholipid bilayer (28), and thus poorly accessible to bulky hydrophilic molecules. For our purposes the matter is not highly relevant, because our goal was to isolate only NHS-SS-biotinylated porin molecules, certainly originating from caveolar domains of the plasma membrane. The application of these proteins to immobilized streptavidin-agarose, followed by elution with dithioerythritol , resulted in a single protein band (Fig. 5*A*). The purified protein was identified as porin by positive staining in Western blots with polyclonal Abs *versus* purified bovine heart mitochondrial porin or with monoclonal Abs *versus* a synthetic peptide reproducing the N terminus of HV-DAC1 (22, 23). This result demonstrates the purification of porin molecules labeled by NHS-SS-biotin, coming from the plasma membrane of intact CEM cells.

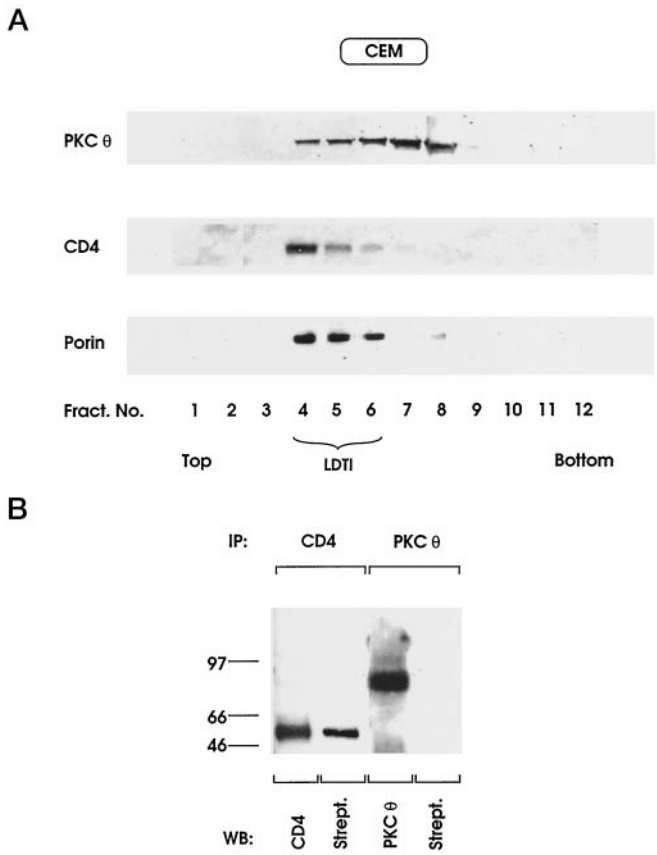

FIG. 4. **CEM cells are not permeable to NHS-SS-Biotin.** *A,* 100×10^6 CEM cells were homogenized in a buffer containing 1% Triton X-100 and subjected to sucrose density gradient centrifugation. 5 μg/fraction were analyzed by Western blotting for PKCθ, CD4, and porin expression. PKCθ, CD4, and porin co-fractionate in fractions 4–7, corresponding to the LDTI buoyant density. *B,* CEM intact cells were labeled by NHS-SS-biotin. LTDI domains were prepared and 20 μg of protein immunoprecipitated (*IP*) with antibodies directed against CD4 or PKCθ. The panel shows detection of immunoprecipitated fractions by the same antibodies (CD4 or PKCθ) or by streptavidin-horseradish peroxidase conjugated (*Strept.*). Only externally exposed biotinylated proteins were detected by the streptavidin-horseradish peroxidase conjugated.

Upon incorporation of this plasma membrane porin into planar bilayers we observed channel activity with properties very similar to those of mitochondrial porin as well as of the other preparations mentioned above. Again, the pores displayed the typical functional features of mitochondrial porin such as the appropriate conductance, voltage dependence, slow and fast kinetics, anion- and cation-selective conductance states (Fig. 6).

We furthermore detected, by patch-clamp in the excised patch configuration on the plasma membrane of CEM cells, an anion-selective channel with characteristics similar to those of the isolated caveolar porin (not shown). The channel is similar to the "maxi-chloride channel" observed in the plasma membrane of several cell types (29, 30).

DISCUSSION

Eukaryotic porin is a much studied but still incompletely understood integral membrane protein very abundant in mitochondria, which forms large pores when reconstituted in artificial membranes (8, 9). Porin isoforms have been identified in humans and their expression in most tissues reported (13). Porin has widely been considered to be localized exclusively in the outer membrane and at contact sites of mitochondria. However, since the first work by Thinnes *et al.* (14), several studies have reported the presence of VDAC in the plasma membrane of lymphocytes (15, 17), respiratory epithelium cells, and astrocytes (16). Its presence in the sarcoplasmic reticulum (31, 32) and endosomes (33) has also been reported. It is particularly relevant for this paper that the major isoform, 31HL/HVDAC1, has been identified by partial sequencing in preparations of caveolae from lung epithelium (7). The notion that VDAC may be present in the plasma membrane has been challenged by Yu *et al.* (18). These authors introduced into three cDNAs for VDAC isoforms sequences encoding for known epitopes, recognized by monoclonal antibodies. Cultured cells were transfected with these constructs, and biochemical, immunofluorescence and immuno-EM techniques were used to localize the products. All three porin isoforms appeared to be located exclusively in mitochondria. Reports associating VDAC with the plasma membrane were tentatively ascribed to nonspecific reaction of antibodies or, in the case of caveolae, to the binding of solubilized VDAC to caveolar components during the preparative procedures. The main aim of this work was to clarify this issue.

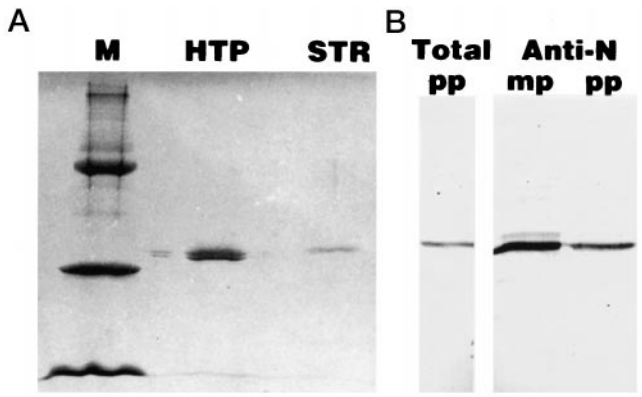

FIG. 5. **Plasma membrane porin purification.** *A,* purification of plasma membrane porin from biotinylated CEM LDTI. SDS-PAGE (reducing conditions) Coomassie Blue staining. *M*, molecular mass markers (from top): bovine serum albumin (68 kDa), carbonic anhydrase (31 kDa), and cytochrome C (12.5 kDa). *HTP*, HTP/celite passthrough obtained from the application of Triton-solubilized material to the chromatographic column; *STR*, protein eluted by the application of a 0.1 M dithioerythritol step to agarose-streptavidin onto which protein from HTP/celite had been adsorbed. *B,* plasma membrane porin is recognized by antibodies *versus* purified mitochondrial porin. Western blot of porin purified from LDTI and immunodetected by polyclonal Abs against purified mitochondrial porin (*Total*) or monoclonal Abs to the N terminus of porin (*Anti-N*) (22). *pp*, plasma membrane porin; *mp*, bovine heart mitochondrial porin.

Labeling of the intact outer face of cultured plasma membrane was used to discriminate plasma membrane protein from other molecules coming from inside the cell. We then applied on the biotinylated caveolar material a mixed purification procedure. The starting material was LTDI (a caveolae-like fraction); in this way we could minimize contamination by most intracellular proteins (19). The first purification step is very selective for porin molecules (20). Next, streptavidin-agarose selected only those porin molecules genuinely coming from the plasma membrane because only these were biotynilated. We are thus confident that the porin molecules purified by this procedure originated from caveolar microdomains in the plasma membrane. We cannot rule out the possibility that porin is present also in other districts of the plasma membrane; our results indicate that it is present in caveolae and caveolae-like material where it is strongly enriched.

The next main point of our work was the identification of porin purified from caveolae. Porin purified from caveolae looked like the same polypeptide isolated from mitochondria. This was shown both by molecular data (identical immunological reactivity, identical chromatographic behavior) and by electrophysiological analysis. When incorporated in planar bilayers, porin from mitochondria and porin purified from caveolae showed indistinguishable properties. The human polypeptide indicated in literature as porin 31HL, HVDAC1, or porin isoform 1 seems thus able to be incorporated both into the plasma membrane and into the mitochondrial outer membrane. This opens stimulating questions about the physiological role of porin in the plasma membrane and about the mechanisms of targeting directing the same polypeptide to two distinct subcellular compartments.

The function of porin in the plasma membrane is unknown. It might serve as the conduit for solute release from potocytotic vesicles. Mitochondrial porin has been shown to catalyze the

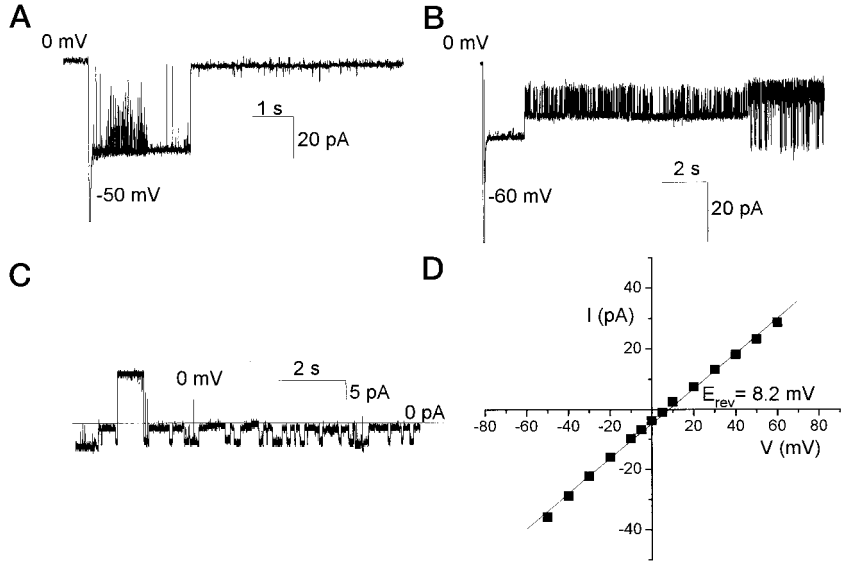

FIG. 6. **Channel activity recorded with porin purified from biotinylated CEM LDTI.** *A,* voltage dependence: a typical trace showing a closure induced by the transition from 0 to −60 mV. Medium, 390:100 mM KCl; membrane, phosphatidylethanolamine. *B,* an example of fast kinetics. Medium, 100 mM KCl; membrane, phosphatidylethanolamine. *C,* gating and transitions between anion- and cation-selective states at zero potential. Conditions are the same as in *A*. *D,* selectivity. A representative I/V plot from an experiment in 390:100 mM KCl. Conductance, 0.58 nS; E_{rev}, 8.2 mV; P_{Cl}/P_K, 1.7.

translocation of DNA through artificial membranes (34), and it might perform a similar function in caveolae. Clearly, the porin in the plasma membrane must generally be in a nonconductive state to avoid dissipation of vital ion gradients. The channel should thus be tightly regulated. Several patch clamp studies have reported a porin-like plasma membrane channel (the "maxi-chloride channel") (16, 29, 30) that is normally closed and opens up only after disruption of the membrane (excised patch mode). We have also observed such a channel in patch-clamp experiments on CEM cells. Following internalization of the caveolae the porin could be open without risking any ionic unbalance.

A second question concerns VDAC targeting to different membranes. Porin insertion into membranes does not require any protein pre-sequence (35). Very few examples of proteins targeted both to mitochondria and to another cellular district have been reported. They showed alternative splicing conferring specific target sequences to expressed proteins (36). For porin, the only hint comes from the recent discovery in the *Drosophila melanogaster* gene of alternative transcripts differing only in the 5′-UTR region of the mature mRNAs (37). Neither of these alternative exons confers to the protein any polypeptide pre-sequence. Whether a similar situation is present also in mammals is unknown, but this hypothesis cannot be ruled out. If it were true, how could different mRNAs coding for the same protein be targeted to different compartments? UTR regions of mRNA have been proposed to be responsible for specific mRNA intracellular localization, a tool for the cell to exert a local translational control (38, 39). Recently evidence was provided for a functional involvement of the 5′ end of *oskar* mRNA, a factor involved in the development of *D. melanogaster*, in the regulation of the mRNA translation (40). Lithgow *et al.* (38) have reviewed the transport of mRNAs to ribosomes close to target subcellular compartments. One can thus hypothesize that different porin 5′-UTRs could contain sequences involved in the targeting of transcripts to localized ribosome pools. This hypothesis is compatible with the data by Yu *et al.* (18), which did not rule out a differentiated targeting of transcripts containing alternative 5′-untranslated sequences.

A second hypothesis on the plasma membrane targeting of porin relies on its affinity for cholesterol. Porin might be present in caveolae and caveolae-like domains simply because these are stations on the way to its final destination(s). Porin binds cholesterol (28, 41, 42), and caveolae are especially rich in cholesterol, playing an important role in its traffic (43). Thus an alternative working hypothesis may be that porin mRNA(s) are translated in the cytosol, and some molecules are transported to the caveolar region by means of caveolin-cholesterol complexes. Next, porin could be relocated to subcellular compartments through the intracellular cholesterol transport routes.

Acknowledgments—We thank F. Thinnes and F. Palmieri for generous gifts of noncommercial antibodies.

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