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Minireview

Evidence for Extra-Mitochondrial Localization of the VDAC/Porin Channel in Eucaryotic Cells

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The expression of bacterial porin in outer membranes of gram-negative bacteria and of mitochondrial porin or voltage-dependent anion channel (VDAC) in outer mitochondrial membranes (OMM) of eucaryotic cells was demonstrated about 15 years ago. However, the expression of VDAC in the plasmalemma (PLM) of transformed human B lymphoblasts has recently been indicated by cytotoxicity and indirect immunofluorescence studies. New data suggest that the expression of VDAC may be even more widespread. Different cell types express porin channels in their PLM and in intracellular membranes other than OMM. The functional expression of these channels may differ in the various compartments since recent experiments have demonstrated that the voltage dependence and ion selectivity of mitochondrial VDAC may be altered by their interaction with modulators. The present paper proposes a unifying concept for the ion-selective channels of cell membranes, in particular, those whose regulation is affected in cystic fibrosis.

KEY WORDS: Porin; VDAC; plasma membrane; cystic fibrosis; chloride channel; sodium channel; long-term potentiation; long-term depression; endosymbiotic theory.

INTRODUCTION

Porins are channel proteins which occur in procaryotes as well as in eucaryotes. The expression of bacterial porins in outer membranes of gram-negative bacteria is well established (Nakae, 1976; Benz, 1988), although there are no data on porins in mycoplasmas or archaebacteria. In eucaryotic cells, porins or voltage-dependent anion channels (VDAC) were first detected in mitochondrial extracts by Schein *et al.* (1976). Since then, different aspects of these channels have been thoroughly investigated and reviewed (e.g., see the multi-author review: *Experientia* **46**, 131–201 1990).

Evidence for the localization of VDAC in eucaryotic cell compartments other than OMM was obtained recently by cytotoxicity and indirect immunofluorescence techniques (Thinnes *et al.*, 1989). There have been other recent observations that VDAC molecules copurify with several receptors. They are found, for example, in the peripheral benzodiazepine receptor complex (McEnery *et al.*, 1991; McEnery, this issue) and in the GABA receptor complex (M. Bureau, University of Liège, personal communication, see below). Furthermore, the B-lymphocyte-derived VDAC "Porin 31HL" copurifies with human transplantation antigens (Thinnes *et al.*, 1989). These data raise the interesting possibility that VDAC may mediate the action of a variety of receptors in various cell membrane compartments.

DIRECT EVIDENCE FOR THE PRESENCE OF PORIN OUTSIDE THE MITOCHONDRIA OF HUMAN CELLS

Porin Molecules at the Surface of H2LCL Lymphocytes

Human porin ("Porin 31HL") was first purified from total membrane fractions of H2LCL lympho-

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cytes (Thinnes *et al.*, 1989). Its sequencing (Kayser *et al.*, 1989) provided the first complete primary structure of a member of this channel protein family from the animal kingdom. The same primary structure was subsequently demonstrated for porin from human muscle membranes ("Porin 31HM") by amino acid sequencing (Jürgens *et al.*, 1991) and for human pituitary gland porin from cDNA analysis (Blachly-Dyson and Forte, 1991).

Different antisera have been raised against "Porin 31HL." Polyclonal antibodies have been prepared against the complete human porin molecule and against a synthetic peptide corresponding to the 19 amino acids at the protein's N-terminus (Thinnes *et al.*, 1989). In addition, a series of eight mouse monoclonal antibodies against human porin have also been prepared (Babel, Thinnes, *et al.*, unpublished results). All of these antibodies have been shown to react with purified porin by immunoelectrophoresis and immunoblotting. Immunoblotting experiments have also been carried out using total membrane fractions of H2LCL cells and membrane fractions treated to minimize the presence of mitochondrial membranes (Babel, Thinnes, *et al.*, unpublished results). With both membrane preparations, the antibodies indicated the presence of "Porin 31HL."

There are two types of evidence that "Porin 31HL" is expressed on the surface of lymphocytes. Cytotoxicity tests using H2LCL cells showed positive reactions using antisera against complete human porin or its N-terminal peptide (Thinnes *et al.*, 1989). In addition, antisera against whole "Porin 31HL" or the N-terminal region of the molecule showed a surface localization in H2LCL cells by immunofluorescence microscopy, whereas pre-immune sera did not show this distribution (Thinnes *et al.*, 1989). More recently, the eight monoclonal antibodies against the N-terminal region of human porin were also tested by immunofluorescence and found to stain the surface of the H2LCL cells (König *et al.*, 1991). These results indicate that the porin molecule, and specifically, its N-terminal portion, is exposed at the periphery of H2LCL cells.

Porin Molecules in Other Nonmitochondrial Eucaryotic Membranes

Indirect immunofluorescence microscopy as well as fluorescence-activated cell sorter experiments indicate that normal B and T lymphocytes also express "Porin 31HL" at their surface in amounts corresponding to those of H2LCL cells (König *et al.*, 1991). Thus, the expression of VDAC in the PLM of human lymphocytes is not related to transformation of the cells. Using the same immunofluorescence-based techniques, we have recently demonstrated the expression of VDAC on the periphery of human epithelial cells (HT29 carcinoma cells) (König *et al.*, 1991). We have also preliminary results (as yet unpublished) indicating the expression of porin in apical membranes of bovine tracheal epithelial cells, rat peripheral myelin, and human sarcolemma.

In addition to the presence of VDAC in PLM, there is evidence for its presence in the sarcoplasmic reticulum of human and rabbit skeletal muscle (Jürgens *et al.*, 1991), the nuclear envelope (König *et al.*, 1991; R, Benz, Univ. Würzburg, personal communication), and in presynaptic vesicles of rat cerebral cortex (kind gift of R. Jahn, Max-Planck-Inst. Psychiatrie) (D. Babel, Max-Planck-Inst. für Experimentelle Medizin, unpublished results).

SUPPORTING EVIDENCE FOR THE PRESENCE OF PORIN OUTSIDE THE MITOCHONDRION

There are several independent pieces of evidence supporting our immunotopological data on the expression of VDAC in the cytoplasmic membrane of cells.

Evidence from Electrophysiology

Patch clamp experiments by Blatz and Magleby (1983) have demonstrated the presence of voltage-dependent, chloride-selective channels in the PLM of embryonic rat skeletal muscle cells, showing large conductances and voltage-dependent gating kinetics similar to those of VDAC. In that study, single-channel recordings were obtained from excised patches of the plasmalemma of cultured rat myotubes. In single-channel experiments in which both sides of the membranes were bathed with 143 mM KCl, the conductance of the "maxi" channel was about 430 pS. The channel was found to be selective for Cl^- over K^+. It was often active at membrane potentials approaching 0 mV, opening and closing spontaneously, whereas raising the membrane potential inactivated the channel. Similar "maxi" channels have since been reported in the PLM of different cells (Schwarze and Kolb, 1984; Kolb *et al.*, 1985; Gögelein, 1988; Schlichter *et al.*, 1990). H2LCL cells show anion

channels of large conductance as revealed by patch-clamp experiments (H.-A. Kolb, Univ. Konstanz, personal communication). We have previously discussed the possibility that porin molecules might be the biochemical basis of the Blatz and Magleby channel (Thinnes *et al.*, 1989; Jürgens *et al.*, 1991). It is, of course, also possible that these maxi-channels are VDAC-like channels with a primary structure different from porin.

Hexokinase Binding

Mitochondrial porin is known to bind the cytoplasmic enzyme hexokinase (Fiek *et al.*, 1982). Parry and Pedersen (1990) have shown recently that brain hexokinase has a propensity to localize at nonmitochondrial receptor sites (also, Arora, Parry, and Pedersen, this issue). It may be that the enzyme is binding to porin present on these nonmitochondrial membranes.

Plasma Membrane Receptors Associated with VDAC-like Molecules

The purified GABA receptor protein complex preparation from mammalian brain contains a component of about 36 kDa. In rat brain, this 36-kDa molecule has been identified as a member of the VDAC family by sequencing cDNA. (M. Bureau, Univ. Liège, Personal Communication).

Other Immunotopological Evidence

An antiserum against rat mitochondrial porin (provided by D. Brdiczka, Univ. Konstanz) was found to label several sites in ultrathin cryosections of rat liver parenchymal cells (N. Roos, Univ. Oslo, Personal Communication). Labelling was predominantly found on the plasma membrane facing the bile canaliculi and on membranes of putative autophagic vacuoles. No labelling was observed on mitochondria, rough endoplasmic reticulum, or the nucleus. The extremely low labelling of the mitochondrial membranes may be due to a low antigen concentration in this organelle.

It should be noted that the above results appear to contradict those of Lindén *et al.* (1984), which were obtained with a different experimental system. In that study, antisera against porin isolated from the OMM of rat liver mitochondria, VDAC was not detected in highly purified rat plasma membranes from bile canaliculi and other liver cell compartments, although there was some indication for the presence of porin in lysosomes.

EXPRESSION OF VDAC IN THE PLASMALEMMA AND THE ENDOSYMBIOTIC THEORY

The expression of VDAC in PLM as well as in the OMM of eucaryotic cells is consistent with the endosymbiont theory of mitochondrial descendance from aerobic purple bacteria belonging to the class of gram-negative eubacteria (Alberts *et al.*, 1989; Gray, 1989). Present-day members of this bacterial group show intimate associations with eucaryotic cells. For example, in the process of forming nitrogen-fixing nodules in roots of legumes, the gram-negative bacterium *Rhizobium* invades and colonizes the cytosol of cortical cells, losing most of its own cell wall during this step. The plasma membranes of the resulting "bacteroids" are surrounded by a membrane which is derived from the host-cell plasma membrane (Alberts *et al.*, 1989). In view of the data discussed in this article, the origin of the outer mitochondrial membrane can be envisioned as the involution of the host cell membrane around an endocytosed symbiont. Thus, VDAC may be derived from the proto-eucaryotic cell's plasma membrane, and not as commonly presumed from the outer envelope of the invading eubacterium.

SPECULATIONS ON THE REGULATION AND FUNCTION OF VDAC IN DIFFERENT CELL COMPARTMENTS

VDAC as a Regulated Channel

Three properties of VDAC are important when considering the roles this channel may play in the plasma membrane of eucaryotic cells. (1) VDAC is voltage-dependent, switching from a fully "open" to more "closed" subconductance states with increasing membrane potentials. The voltage-dependence of porin channels can be modified by macromolecular modulators (Benz *et al.*, 1988; Holden and Colombini, 1988). Thus, VDAC should not be regarded as an unspecific sieving pore but as a channel which may be regulated by interaction with modulators. (2) VDAC is not ion specific but it is ion selective, able to change from anion to cation selectivity in correlation with the state of channel opening (Benz *et al.*, 1990).

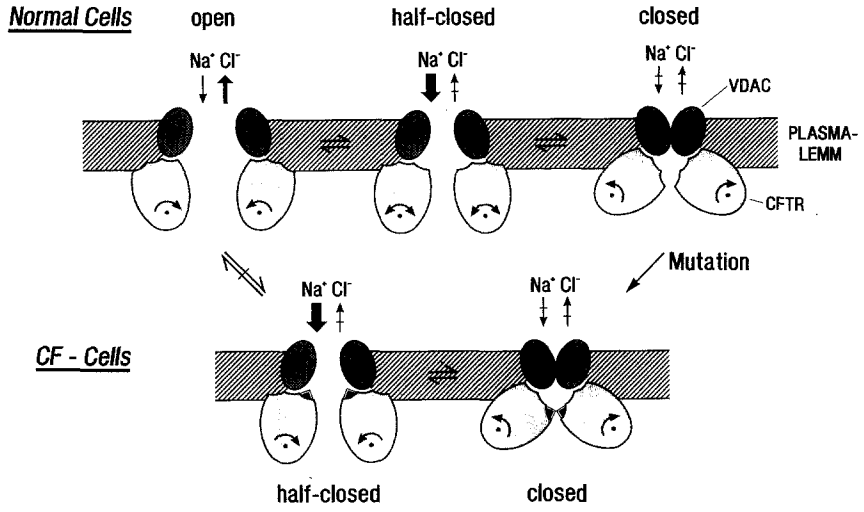

Fig. 1. Refined flip-flop model of the postulated ion channel complex involved in the disturbed chloride and sodium fluxes across the plasmalemma of cells affected by cystic fibrosis (CF). In resting cells plasmalemma integrated VDACs are kept closed by interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). Conformational changes of the CFTR during cell stimulation in normal cells allow full opening of VDACs to their "open" anion (Cl^-) selective conductance state. In CF-cells showing mutated forms of the CFTR molecule, only a "closed" cation (Na^+) selective subconductance state of VDAC can be reached, leading to increased Na^+ influx but suppressed Cl^- efflux of CF-cells. This model was first presented at the Third Symposium Cystic Fibrosis, Berlin, April 26–28, 1991.

(3) Purified VDAC in artificial membranes never closes totally, even when membrane potentials are applied that are higher than those physiological for mammalian cells. These facts suggest that biochemical regulation of VDAC may take place when it is incorporated into the plasmalemma of cells (Thinnes *et al.*, 1991).

VDAC as the CF-Linked Channel of the Plasma Membrane

A basic defect in cystic fibrosis (CF) is that the PLMs of different cells are impermeable to chloride (Frizzell and Cliff, 1991; McPherson and Dormer, 1991). Also, increased Na^+ conductance is observed (Boucher *et al.*, 1988), part of which is mediated by amiloride-insensitive cation channels which are not yet defined on the molecular level (Jorissen *et al.*, 1991). "Porin 31HL" and the chloride channel affected in CF share several traits (Thinnes *et al.*, 1990, 1991): expression in the plasmalemma of different cells, molecular masses of around 30 kDa, high conductance values when incorporated in artificial membranes, ability to be modified by DIDS, and subject to regulation by modulators. On this basis, we have formulated (Thinnes *et al.*, 1990, 1991) and since refined a two-component flip-flop model of a postulated VDAC-containing chloride channel complex misregulated in CF. According to this model (Fig. 1), in resting cells, VDAC in the PLM is kept closed by interaction with the cystic fibrosis transmembrane conductance regulator (CFTR). In normal cells, cell stimulation induces conformational changes in the CFTR, allowing full opening of VDAC to the "open" anion (Cl^-) selective conductance state. In CF cells, the CFTR has mutated and only allows a "closed" cation (Na^+) selective subconductance state of VDAC. In this way, both the increased Na^+ influx and suppressed Cl^- efflux of CF-cells may be explained.

VDAC in the Endoplasmic Reticulum

We previously hypothesized (Thinnes *et al.*, 1991) that, in immunocompetent cells, MHC-coded proteins homologous to the CFTR (Parham, 1991) might regulate VDAC of the endoplasmic reticulum (ER). Recently, protein-conducting channels of pancreatic rough microsomal vesicles were studied after fusion of the vesicles to planar lipid bilayers in 50 mM salt solutions. Single-channel conductance steps of 220 pS were observed (Simon and Blobel, 1991), data fitting surprisingly well to values shown by VDAC in arti-

ficial membranes (4200 pS in 1 M KCl). In analogy to our model for the PLM chloride channel complex, the presumed VDAC of ER may be kept closed by a CFTR-like modulator (Parham, 1991). (This would answer a previous objection to a wide-open porin-like channel existing in the ER by Simon *et al.*, 1989.) Conversely, the porin channel in such a complex may be opened by its interaction with ribosomes.

Association of Porin-Like Molecules with PLM Receptors

VDAC copurifies with receptors in two known cases (see above), supporting the concept of different receptors linked to a single ion channel (Hamill *et al.*, 1983). *In vitro*, purified VDAC exhibits two highly conducting substates; *in vivo*, VDAC must be regulated (especially in the PLM) and likely exhibits additional functional states. We have preliminary evidence that VDAC occurs in presynaptic membrane vesicles (see above) and we suspect that VDAC may be part of receptor channel complexes involved in long-term potentiation/depression (Goldman *et al.*, 1990). Our approach to this fascinating problem will be to search for and to characterize synaptic VDAC modulators.

CONCLUSIONS AND PERSPECTIVES

There is strong evidence for multi-compartment localization of VDAC in eucaryotic cells. The VDAC channel in the plasma membrane must be regulated. By interacting with modulators, VDAC can display altered voltage dependence and ion selectivity. Thus, the function of VDAC may vary with its location. In the plasmalemma of epithelial cells, we propose that VDAC may be part of a two-component channel complex whose other component, CFTR, is altered in cystic fibrosis.

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