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Large Cation-Selective Pores from Rat Liver Peroxisomal Membranes Incorporated to Planar Lipid Bilayers

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Summary. Fusion of a highly purified fraction of rat liver peroxisomal membranes to planar lipid bilayers incorporates large, cation-selective voltage-dependent pores. The P_K/P_{Cl} ratio of these pores, estimated in KCl gradients, is close to 4. The pores display several conductance states and spend most of the time open at voltages near 0 mV, closing at more positive and negative voltages. At voltages near 0 mV the most frequent open state has a conductance of 2.4 nS in 0.3 M KCl. At voltages more positive and more negative than 10 mV the most frequent open state displays a conductance of 1.2 nS in 0.3 M KCl. With these results pore diameters of 3 and 1.5 nm, respectively, can be estimated. We suggest that these pores might account for the unusually high permeability of peroxisomes to low molecular weight solutes. Fusion also incorporates a perfectly anion-selective, two-open states channel with conductances of 50 and 100 pS in 0.1 M KCl.

Key Words peroxisome · fusion · planar bilayer · pore

Introduction

The properties and specific differentiation of the membranes in cell organelles are largely responsible for their structural and functional properties. Experimental evidence shows that similar to plasma membranes, organelle membranes have sites of recognition, enzymes, cytochromes and specific proteins. They also have special mechanisms involved in ionic and neutral solute translocation, such as the large anion-selective pores present in the outer membrane of mitochondria and chloroplasts [2] and the calcium and potassium channels present in the sarcoplasmic reticulum from vertebrate skeletal muscle [9, 17, 23]. A better knowledge of the distribution and properties of these channels or pores is helpful in understanding the control of molecular traffic inside the cell.

Peroxisomes constitute a family of subcellular organelles characteristic of eucaryotic cells. Their function is only partially understood, particularly for animal peroxisomes [6, 8, 25]. Their involvement in various aspects of lipid metabolism has been stressed, particularly after they were shown to oxidize fatty acids via a β-oxidative pathway [10], and more recently, because the metabolic errors recognized in a genetic disorder found in humans have been associated to the complete or almost complete absence of peroxisomes [8, 22].

On the basis of indirect evidences it is known that several different molecules pass across the peroxisomal membrane, but the molecular basis of this translocation is not understood for any of them. It is well known that the peroxisome membrane is unusually permeable to low molecular weight solutes [6], but CoA-thioesters fatty acid do not cross it freely [12, 24]. Proteins incorporate post-translationally into peroxisomes from the cytoplasm by mechanisms not well established [11]. In addition, proteins easily leak in vitro from peroxisomes [13], a phenomenon responsible for at least part of the enzymes present in high-speed supernatants from tissue homogenates and for the presence of peroxisomal ghosts in particular fractions observed by electron microscopy [6, 13].

To gain insight into the mechanisms responsible for the peculiar permeability properties of peroxisomes, studies were performed taking advantage of methods now available for the isolation of these organelles [27, 28] and their membranes [7, 16] and also the development of techniques that allow to incorporate membrane fragments to planar lipid bilayers, rendering their permeability properties susceptible to electrophysiological analysis [18].

We report here some results which constitute initial findings in the study of peroxisomal membrane permeability using the fusion method developed by Miller [19]. The fusion of peroxisomal membranes to planar lipid bilayers results in the incorporation of pores, slightly cation selective. The presence of these channels could well account for some of the permeability properties of peroxisomes.

Materials and Methods

Membrane Preparation

Peroxisomes were isolated from normal male rat liver by isopycnic equilibrium in metrizamide gradients loaded with a peroxisome-rich fraction obtained by differential centrifugation [5, 12, 27]. Peroxisomes were more than 95% pure as estimated from catalase specific activity. The remaining 5% protein was equally distributed among endoplasmic reticulum and mitochondria as estimated from measurements of NADPH cytochrome *c* reductase and glutamate dehydrogenase, respectively [5, 15]. Peroxisomal membranes were prepared treating the peroxisomal fraction with 10 mM sodium pyrophosphate, pH 9.0 [16] or 0.1 M sodium carbonate, pH 11.5 [7]. The peroxisomal fraction from the metrizamide gradient was diluted 10-fold in 0.25 M sucrose and centrifuged for 20 min at 28,000 rpm in a #65 Beckman rotor. The pellet was taken to the same volume of the sucrose dilution with either pyrophosphate or carbonate buffer and after 30 min in ice with gentle stirring the membranes were sedimented 60 min at 50,000 rpm in the same rotor. The pellet surface was gently rinsed with distilled water and the membranes were suspended in 0.4 M sucrose by sonication.

Bilayer Setup and Electrical Measurements

Bilayers were formed according to Mueller et al. [20] in a hole (300 μm diameter) made on a Teflon® partition separating two compartments. Both compartments were filled with 100 mM KCl buffered with 5 mM MOPS-K, pH 7.0. Lipids consisted of a 10 mg/ml solution of brain phosphatidylserine-brain phosphatidylethanolamine 1 : 1 (Avanti Polar, Birmingham, Ala.) in *n*-decane. After bilayer formation the KCl concentration on the *cis* chamber was increased by adding an aliquot of 3 M KCl, to favor fusion. Peroxisomal membranes (3 to 10 μg/ml final protein concentration) were added into the *cis* side. Channel incorporation was detected as the appearance of square fluctuations in current. Current was measured with a two-electrode voltage clamp [1]. The *cis* chamber was connected to a voltage pulse generator and the opposite *trans* chamber 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 is virtual ground. The current was low-pass filtered, amplified and stored in tape.

Single-channel currents were estimated from the amplitude of discrete current jumps. Data were analyzed by hand.

Results

Fusion of Peroxisomal Membrane Incorporates Channels in Planar Bilayers

The addition of peroxisomal membrane vesicles to the *cis* chamber, in the presence of an osmotic gradient, induces stepwise increases in bilayer conductance, as illustrated in Fig. 1*A*. The Figure shows a record of membrane current obtained in the presence of a KCl gradient at zero applied voltage. At the time labeled with an arrow an aliquot of peroxisomal membrane vesicles was added to the *cis* chamber, under stirring. As shown, soon after the addition, an upward jump in the current is observed, followed by current fluctuations. The appearance of a positive current at zero applied voltage indicates the insertion of a cation-selective conductance pathway. With better time resolution, it can be shown that the current fluctuations seen in Fig. 1*A* are actually due to the opening and closing of channels, as illustrated in Fig. 1*B*. Channel insertion was observed both with the PP_i and the carbonate isolation procedures. In what follows, we describe the most salient features of these channels.

Large, Cation-Selective Channels

The records of current fluctuations shown in Fig. 1*A* and *B*, indicate that the channels inserted into planar bilayers upon fusing peroxisomal membranes

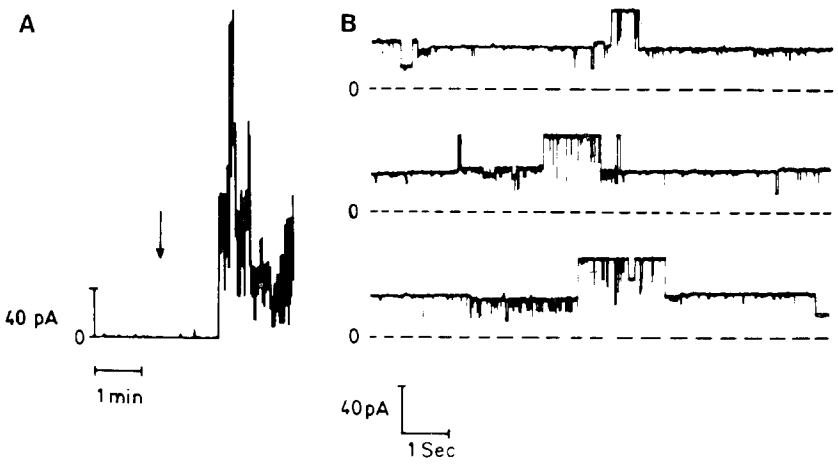

Figure 1. Fusion of peroxisomal membrane vesicles with planar lipid bilayers. Membranes were formed in symmetrical 0.1 M KCl. Then the KCl concentration in the *cis* side was raised to 0.4 M; vesicles were added to the *cis* chamber, under stirring. (*A*) Experimental record of current increase in a bilayer. The applied voltage was 0 mV. (*B*) Experimental record of channel current fluctuations from an experiment similar to that shown in *A*, with better time resolution. The dotted line indicates zero-current level