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Single-channel analysis of a large conductance channel in peroxisomes from rat liver

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Native membranes and Triton X-100 solublized integral membrane proteins of peroxisomes from rat liver were reconstituted in liposomes. With the patch clamp technique, a channel was detected with a conductance of 420 ± 30 pS and a P_K/P_{Cl} of about 3. The channel in native membrane fractions was weakly voltage dependent, residing most of the time in an open state with the possibility to shift to different substates. Solubilization changed the kinetic properties. The channel became strongly voltage dependent and closed at voltages negative to −20 mV. The estimated diameter of the channel is about 1.7 nm and might explain, at least partially, the permeability properties of the peroxisomal membrane.

Introduction

Isolated rat liver peroxisomes are permeable to sucrose [1,2] and to a variety of other small molecules including substrates and cofactors for intraperoxisomal enzymes [3,4]. The sucrose permeability of the peroxisomal membrane is an unusual property that is shared by the mitochondrial outer membrane, but not by most other subcellular membranes. The mitochondrial outer membrane contains a channel-forming protein, porin, which allows the diffusion of solutes with molecular weights lower than 4000–6000. The porin channel is weakly anion selective and has a conductance in the order of 500 pS. Porins are also found in the outer membranes of chloroplasts and Gram-negative bacteria (for review, see Ref. 5).

Experiments from one of our laboratories have indicated that the membrane of rat liver peroxisomes also possesses a pore-forming protein, which allows the passage of solutes with molecular weights of up to 800 at least [4,6]. Labarca et al. [7] described the presence of a weakly cation-selective large conductance channel in peroxisomal membrane fragments fused with planar lipid bilayers. Our previous observations and those of Labarca et al. are most probably the expression of the behavior of a same peroxisomal integral membrane protein. The protein remains to be identified and purified.

In the current contribution we present a detailed electrophysiological analysis of the behavior of the peroxisomal channel at the single-channel level. Peroxisomal native membrane fragments and detergent-solubilized peroxisomal integral membrane proteins were reconstituted into liposomes and the characteristics of the channel in its native membrane environment and after solubilization were studied with the patch-clamp technique. The experiments confirm the presence of a weakly cation-selective large conductance channel in the peroxisomal membrane, and show that some of its characteristics change upon solubilization. The channel is clearly distinct from the porin present in the outer mitochondrial membrane as well as from the weakly cation-selective large conductance channel that was recently also found in mitochondria [8]. The submitochondrial localization and the role of the latter channel remain unknown. The estimated diameter of the peroxisomal channel is sufficient to explain the non-selective permeability of isolated peroxisomes to substrates, products and cofactors for intraperoxisomal enzymes.

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Material and Methods

Purification of peroxisomes and preparation of peroxisomal membrane fractions

Peroxisomes were purified from livers of male clofibrate-treated rats by a combination of differential centrifugation and isopycnic centrifugation in isoosmotic self-generating Percoll gradients as described earlier [4,9]. Calculated on a protein basis, the mitochondrial contamination of the peroxisomal preparations was approx. 8%. Peroxisomal membranes were prepared by sonication of the purified organelles in 10 mM pyrophosphate buffer pH 9 exactly as described previously [4]. Sonication in hypotonic pyrophosphate buffer releases the matrix proteins and most of the peripheral membrane proteins. The obtained membrane pellet was suspended in 10 mM Mops buffer pH 7.2, containing 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF and 0.1% (v/v) ethanol and the membranes were stored in liquid nitrogen before reconstitution in liposomes. In another series of experiments the membrane pellet obtained after pyrophosphate treatment was resuspended in the same buffer solution as described above except that the solution contained 0.5% (w/v) Triton X-100 and the integral membrane proteins were solubilized exactly as described earlier [4]. The solubilized proteins were stored in liquid nitrogen before reconstitution into liposomes.

Reconstitution of channels in freeze-thaw liposomes

Blank freeze-thaw liposomes (without reconstituted channels) were prepared as follows. 200 μl of a chloroform solution, containing 5 mg phosphatidylcholine, 5 mg phosphatidylethanolamine, 2 mg phosphatidylserine and 2 mg cholesterol per ml of chloroform, was dried (in a warm water bath at 37°C) as a thin film on the bottom of a test tube by means of a nitrogen stream. 200 μl of an aqueous solution containing 100 mM KCl, 1 mM $CaCl_2$ and 5 mM Hepes brought to pH 7.5 with Tris were added. The dried lipid was allowed to hydrate for 30 min at 37°C. The suspension was vortexed for 2 min and sonicated with a probe sonicator (Analis probe sonicator 20 micron peak to peak) for four times 5 s with 5-s time intervals at 2–4°C. The obtained small unilamellar vesicles were frozen in liquid nitrogen in aliquots of 10 μl and stored. Before use, the samples were thawed at room temperature for 10 to 15 min, yielding liposomes.

In order to reconstitute peroxisomal native membrane fragments, aliquots (30 to 100 μl) of the pyrophosphate-treated peroxisomal membrane fraction, containing approx. 300 μg of protein, were added to the aqueous solution used for the hydration of the dried lipids.

Triton X-100 solubilized integral membrane proteins were reconstituted by adding aliquots (50 to 70 μl containing approx. 30 μg of protein) of the solubilized proteins directly to the unilamellar vesicles. The suspension was kept for 2 h at 2–4°C and freeze-thawed, yielding liposomes. In some experiments 100 mg wet Bio-Beads SM-2 were added together with the solubilized proteins in order to adsorb the detergent [10]. Before freeze-thawing the beads were removed by sedimentation.

As an additional control Triton X-100 was added to the unilamellar vesicles in the absence of protein. The final Triton X-100 concentration in these experiments was 0.25% (w/v), a concentration approximately twice the maximum concentration present during the reconstitution of the solubilized proteins.

Incorporation of gramicidin D was achieved by adding a small volume (50 μl) of a gramicidin solution (3.4 μg gramicidin/ml) to the liposomes. The suspension was allowed to equilibrate for 2 h at room temperature.

Single-channel recording

10 μl of the thawed liposome suspension was diluted in a bath containing the aqueous solution used for the preparation of the liposomes. Dilution resulted in an increase in surface blebbing and in the formation of liposomes with a diameter as large as 50 μm [11]. The liposomes were allowed to settle at the glass bottom of the bath. The bath was flushed to remove the monolayer formed at the surface of the aqueous solution and the vesicles not sticking to the bottom.

Patch pipettes were made from pyrex glass capillaries on a home-made two-stage microelectrode puller and microforge. The initial resistance of the pipette was 10 to 20 MΩ. Pipettes were sealed on the outer membrane of the liposomes. Patches were excised by short exposure of the tip of the pipette to the air. Patches without channel-forming activity had a resistance of 23 ± 15 GΩ ($n = 215$, $\pm$S.D.). The success rate for patch formation was more than 90%. The patches remained stable during prolonged clamping at potentials ranging from -100 to $+150$ mV.

Unless indicated otherwise, both pipette and bath were filled with the same aqueous solution mentioned above. Pipette solutions were filtered through a Millipore filter (pore diameter of 0.2 μm). The potential values indicate the pipette potential with respect to bath potential. Outward current is defined as positive charges flowing out the pipette. The experiments were carried out at room temperature.

Single-channel currents were measured with a List LM/EPC5 patch-clamp amplifier. Data were stored on a FM magnetic tape recorder. After filtering, the data were digitized and analyzed by computer (PDP 11/23 DEC).

Suitability of the reconstitution method. Control experiments

Freeze-thaw liposomes may be complex multilamellar structures [11]. In order to verify whether the

patches were of a single bilayer structure and not a plug of undifferentiated lipid material, gramicidin D or Triton X-100, which have channel-forming activity in single bilayers [12,13], were incorporated in liposomes. With 200 mM KCl present both in the pipette and bath solution, clean square current transitions of single gramicidin channels could be observed in isolated patches, indicating the presence of a bilayer. The gramicidin channel had a conductance of about 20 pS, and open times in the order of seconds. This behaviour is similar to the characteristics described previously [12]. In a similar way, we were able to record channel-forming activity of Triton X-100. The channels had a maximum conductance of 205 ± 60 pS ($n = 3$, ±S.D.) and remained in an open state for more than 95% of the time when clamped at potentials between −100 and +100 mV. These results are in good agreement with those reported by Schlieper et al. [13].

The channel-forming activity detected with gramicidin or Triton X-100 indicated that the patches consisted of a single bilayer, and therefore demonstrates the suitability of the reconstitution method.

Results

Reconstituted native peroxisomal membrane fragments

General description

In a first series of experiments vesicles were analyzed in which native membrane fragments were incorporated. Active channels were found in about 10% of the patches. Fig. 1 presents examples of current records at four different voltages from an experiment in which a patch was subjected to successive clamps at increasing positive or negative potentials.

At voltages less than 100 mV the current was for more than 95% of the time at a given level. This level was proportional to the voltage (indicated in Fig. 1 as the main level, M). Occasionally, short transitions to lower levels occurred. The probability per unit time of such transitions at +20 mV was low ($0.47 \pm 0.31\ s^{-1}$ for $n = 3$, ±S.D.) and increased with voltage. Rarely, i.e. in less than 1% of the cases, transitions between smaller current levels were also detected. For clamps at 100 mV and at 150 mV (negative or positive) transitions to smaller current levels became more frequent and the residing times at smaller current levels were longer (seconds to even minutes). The shift to such long duration lower level occurred with a delay which was variable and ranged from a few seconds to minutes. If the shift to a lower level persisted for minutes, the original level could only be regained by lowering the voltage gradient. In some cases a decrease by 50 mV was sufficient. In other cases the voltage had to be reduced to zero mV for 5 to 30 s.

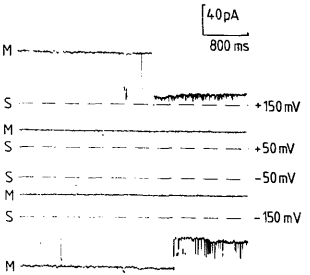

Fig. 1. Current records from a patch with a single peroxisomal channel. The smallest detected current level (S) is indicated. The channel resided at a main current level (M) with sporadic transient transitions to different smaller current levels (±50 mV). For clamps at larger voltages (±150 mV) the residing time at smaller current levels became longer, ranging from seconds up to minutes. Records were low-pass filtered at 500 Hz and digitized at 2 ms. Conditions: pipette and bath solution contained 100 mM KCl, 1 mM $CaCl_2$ and 5 mM Hepes brought to pH 7.5 with Tris.

In the example of Fig. 1 the main level corresponds to a conductance of 420 pS (after leak correction). The same value was found in five other patches. In 17 patches the behaviour of the channels was similar but the main current level was higher. The distribution of the currents or conductances revealed the existence of groups. This is illustrated in Fig. 2a by the different slopes of the current-voltage relationships obtained from the mean values of the current at each voltage. In Fig. 2b the distribution is given of the conductance calculated for each individual experiment. The values of the conductances could be considered multiples of a basic unit of 420 pS. From this observation it was concluded that these patches contained two, three or four channels.

Significance of short transitions. Existence of substates

A more detailed analysis of the current levels attained during the short transitions is given in Fig. 3. The patch was clamped at +100 mV and showed a main conductance level of 420 pS. Fig. 3a shows the observed current jumps in order of increasing magnitude. At least eight different current levels could be discerned some of which correspond to peaks found in the current amplitude histogram of the same current record which is presented in Fig. 3b. The largest peak at the right corresponds to the main current level (M) and amounts to 48.8 pA at +100 mV. The smallest level (S) was 5.2 pA. The different current levels were situated between the smallest and the main current level. The

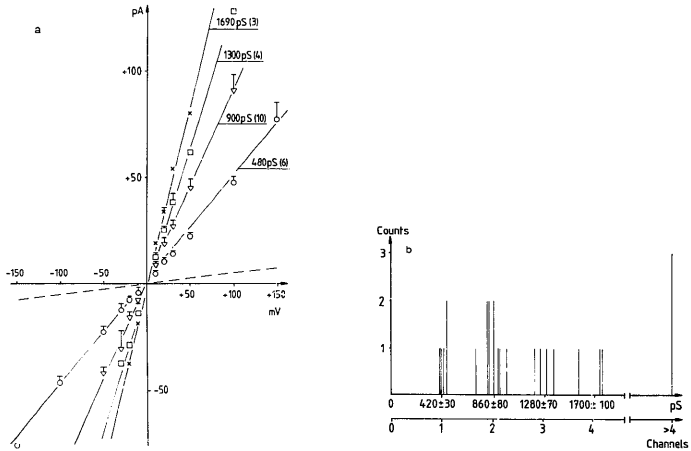

Fig. 2. Current-voltage relationships of the main current levels of 24 experiments (Fig. 2a). The main current levels were determined with current amplitude histograms of current records of 1 to 5 min. The obtained values at each potential were grouped in four groups (circles, squares, triangles and crosses) and averaged (±S.D.) for each group. The data were fitted to straight lines. The number of experiments in each group is indicated. The dotted line represents the average leak found in blank patches (50 pS). The distribution of the conductances after leak correction is illustrated in Fig. 2b. The conductances of the experiments in each group were averaged (±S.D.). Conditions as in Fig. 1.

smallest detected state probably corresponds to the closed state of the channel (5.2 pA at +100 mV or 52 pS). A conductance of 52 pS or a resistance of about 20 GΩ is well within the range of the mean value of 23 ± 15 GΩ (±S.D.) for 215 patches in which no channels were detected. The simplest way to explain the

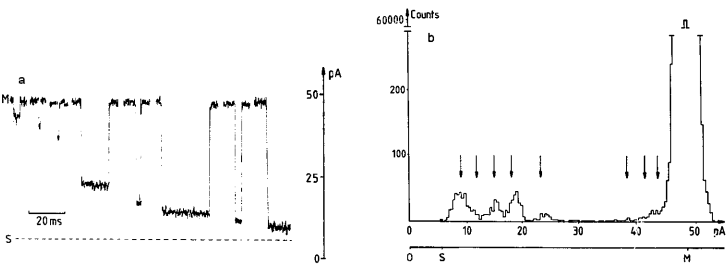

Fig. 3. Fig. 3a illustrates the presence of different current levels between the main open and the closed level detected in a single channel clamped at +100 mV. At least eight different current levels were discerned. A current record of 2 min of the same experiment was used to construct the current amplitude histogram presented in Fig. 3b. The smallest detected current level is indicated at the left (S) and is presumably the closed state of the channel. The peak at the right is the main open state (M). The smaller current levels are situated between these two states. The arrows indicate the current values found in Fig. 3a. Records were filtered at 2.5 kH and digitized at 50 μs. Conditions as in Fig. 1.

result of Fig. 3 is to assume the existence of one channel which most of the time resides in the open main level, and occasionally shifts to a partially closed state or substate.

Another possibility is the assumption of several independent channels. Two possible explanations could then be offered for the short transitions to lower levels: they are due either to the simultaneous closure of one or more channels or to the closure in each case of a different channel. (1) If the transitions to lower levels were due to the simultaneous closure of more than one channel, a high closing probability would be required. Such a condition is contrary to the observed results. The probability for closure to any level was very low (0.47 s^{-1} at +20 mV). If this value is taken as an (overestimated) approximation of the probability of closing of one channel in an interval of 1 s, the probability of simultaneous closing within 1 ms (the time resolution of our measurements) of two independent channels is vanishingly small ($2.2 \cdot 10^{-7}$). (2) If the transitions were due to the closure of a single channel, the main open state should be equal to the sum of all individual channels. Inspection of the different levels demonstrates that this is not the case. It is therefore concluded that the short transitions are due to changes to substates.

Probability of the main open and closed state

In the six experiments in which only one channel (i.e., one main current level) was present, amplitude histograms were constructed at different voltages, and the probabilities were computed. When the analysis is restricted to periods before the channel shifted to a long-live substate (i.e., substates with duration longer than 10 s), the results can be summarized as follows (Fig. 4). The probability of the main open state is high (practically 1.0) at small potential differences, but decreases slightly at more extreme potentials. In all cases the probability of the closed state is practically zero.

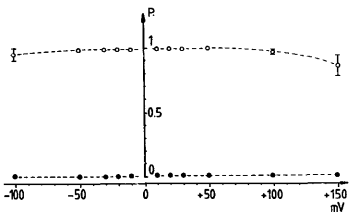

Fig. 4. The probability of the main open state (open circles) and of the closed state (dots) was determined while the channel resided in the main open state with transient transitions to substates (absence of long-live substates). The channel was clamped at increasing positive or negative potentials. The probabilities were derived from amplitude histograms of a current record at each potential. For small potential differences (from −50 to +50 mV) the duration of the current records used varied from 30 s to 5 min. At extreme potential differences, the channel may switch to long-live substates (up to several minutes). In order to avoid this phenomenon clamps at these potential were shorter (up to 2 min at +100 mV). The data are from six single-channel experiments (±S.D.). Conditions as in Fig. 1.

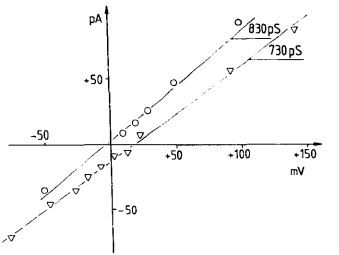

Fig. 5. Selectivity of the peroxisomal channel. The circles represent the current-voltage relation of the main open state in symmetrical conditions (see Fig. 1). The data were fitted to a straight line with a conductance of 830 pS (2 channels). The triangles represent the current-voltage relation when the KCl concentration in the bath was raised to 500 mM. The reversal potential shifted to about +20 mV, yielding a P_K/P_{Cl} ratio of about 3.

Conductance selectivity

The selectivity of the channel was determined by increasing the KCl concentration in the bath to 500 mM. In Fig. 5 an example is presented of the current-voltage relationship in these asymmetrical conditions. This patch contained two peroxisomal channels. The reversal potential of the main open state shifted to about +20 mV at the most diluted side, indicating a weak selectivity of the channel to cations over anions. For three experiments, an average reversal potential of +17.1 ± 2.6 mV (±S.D.) was obtained. The degree of selectivity was calculated with the Goldman-Hodgkin-Katz equation. P_K/P_{Cl} was about 3, which means that the peroxisomal channel conducts about three times more potassium than chloride.

Reconstitution of solubilized membrane proteins

General description

Channel activity in liposomes in which solubilized membrane proteins were incorporated showed remarkable differences with the results described in the previous section. An example is given in Fig. 6. At positive voltages, the activity was characterized by a pronounced flickery behaviour, with high frequency oscillations and without any clear constant level. In amplitude histo-

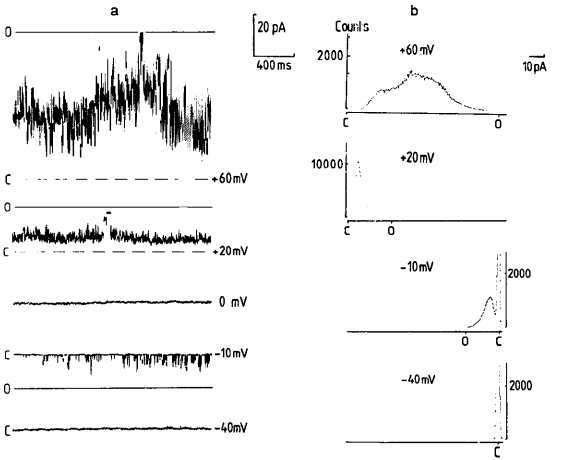

Fig. 6. Fig. 6a presents current records of solubilized peroxisomal channels at different potentials. Such records with a duration of 1 to 5 min were used to construct current amplitude histograms as presented in Fig. 6b. The closed state (C) and the maximum detected current level (O) are indicated. The leak at positive potentials was estimated by linear extrapolation from the measured leak at negative voltages. The maximum current level was determined with current amplitude histograms as presented. Records were low-pass filtered at 1 kHz and digitized at 1 ms. Conditions: pipette and both solution contained 100 mM KCl, 1 mM $CaCl_2$ and 5 mM Hepes brought to pH 7.5 with Tris.

grams no clear peaks could be detected. At less positive voltages, there was a tendency to stay less open, but the flickery behaviour continued. The amplitude histogram became narrower (+20 mV). At negative potentials the channel openings decreased and stopped completely at voltages negative to −20 mV at which the channel remained closed (−40 mV). These phenomena occurred in all patches examined ($n = 6$), indicating not only a strong asymmetrical dependence of the channel on potential, but also a preferential direction of incorporation of the channel in artificial membranes. The use of Bio-Beads SM-2 in order to adsorb selectively the detergent Triton X-100 [10] during reconstitution did not change the above described properties.

Conductance

When the maximum current level observed in the amplitude histograms was plotted as a function of the potential, a linear relationship was obtained (Fig. 7). Intersection with the abscissa occurred at about 0 mV. The slope of the relation was 860 pS in one experiment and 1290 ± 180 pS (±S.D.) in five other experiments. These conductances appear to be integral multiples of the single channel conductance of 420 pS as observed in native peroxisomal membrane fragments. The patches were thus considered to contain two or three channels.

Selectivity

Selectivity of the solubilized channel was determined after increasing the KCl concentration in the bath from 100 to 500 mM. The reversal potential shifted to 18 ± 5 mV ($n = 3$, ±S.D.), indicating a weak selectivity of the channel to cations over anions. The degree of selectivity was calculated with the Goldman-Hodgkin-Katz equation, and a P_K/P_{Cl} of about 3 was found.

Kinetics

From the description of the channel behaviour, it is clear that the channels rarely appeared in the fully open state. Depending on the voltage the current flickered between different open levels at positive voltages and gradually changed to the closed state at negative voltages. In order to measure the probability of one channel being in the closed state, it is needed to know the number of channels in the patch. This value was estimated from the measured conductance levels. The closed

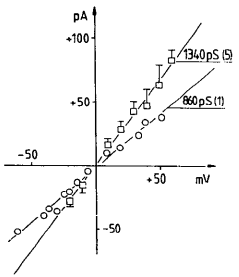

Fig. 7. Current-voltage relationships of the maximum current levels that were detected at different potentials in six different patches. The maximum current level was always determined using current amplitude histograms constructed with current records at a fixed potential. Above −20 mV, current records of at least 1 min were used. Below −20 mV maximum current levels were determined before the channels closed. Such closure occurred when they were clamped to the negative test potential starting from positive holding potentials. The maximum current levels at each potential were divided in two groups (circles and squares), averaged (±S.D.) and fitted to a straight line. Leak was subtracted. The conductances were 860 and 1340 pS. Conditions as in Fig. 5.

probability was determined using current amplitude histograms of records of 1 to 2 min. The closed probability was then calculated using a binomial distribution and assuming an independent behaviour of the channels. The results from six experiments are presented in Fig. 8. The closed probability was strongly voltage dependent, and increased from less than 10% at potentials positive to +20 mV to more than 90% below −20 mV.

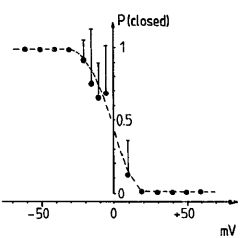

Fig. 8. Closed probability calculated for a single solubilized peroxisomal channel. The closed probability in a patch was determined from current amplitude histograms of records of at least 1 min at each potential. The number of channels was determined, and they were assumed to be independent. The closed probability of a single channel was calculated with a binomial distribution. The dots are mean values of the closed probability (±S.D.) for six experiments.

Discussion

In the present report we have analyzed channel activity induced by incorporation of peroxisomal native membrane fragments or peroxisomal Triton-solubilized membrane proteins in lipid membranes. Single-channel conductance was in the order of 420 pS and a selectivity of P_K/P_{Cl} of about 3 was found. Channel kinetics was different for the native membrane and solubilized fraction. Channels derived from the native membrane fraction, subjected to moderate potential gradients, were mostly in the open state and occasionally shifted to a short-duration substate. At larger potentials, the shift to a substate was of longer duration. Channels derived from the solubilized fraction had a pronounced flickery behaviour, and tended to close at negative pipette voltages.

The channel is different from the channel induced by Triton X-100 as such. The detergent-induced channel in our hands had a single-channel conductance of 205 pS and a closed-state probability not dependent on potential. The channel induced by the solubilized peroxisomal proteins had a conductance of 420 pS and closed at negative potentials. The properties of the peroxisomal channel are different from those of other large conductance channels presently known in rat liver. In mitochondrial membranes two large conductance channels have been described [5,8,14–16]. One of them, the voltage-dependent anion channel (porin) can be excluded on the basis of its anion selectivity. The other channel is cation selective but differs from the peroxisomal channel in two aspects: first it shows four conductance levels; second the open probability of these conductance levels is strongly voltage-dependent and totally different from the present channel. Control experiments were carried out under identical conditions with a total mitochondrial membrane fraction from rat liver. In this mitochondrial membrane fraction, channels with similar characteristics to those described for the mitochondrial porin were observed. Similar channels were detected after reconstitution of peroxisomal fractions (in both native membrane fragments and solubilized peroxisomal proteins) in 4 to 5% of the patches with channel forming activity. These findings are explained by the small contamination of our peroxisomal fraction with mitochondria. Gap junctions from rat liver have a conductance of 150 pS [17] which is clearly lower than the conductance of the peroxisomal channel. Moreover, gap junctions are presumably closed in the presence of 1 mM Ca^{2+}.

The conductance of the peroxisomal channel in our hands is about 420 pS in 100 mM KCl. Labarca et al. [7] reported a channel in peroxisomes from rat liver

with a conductance of 1.2 nS and 2.4 nS in 0.3 M KCl: the largest conductance was most frequently observed at potentials near −10 mV, while the smaller conductance was mainly present at potentials more negative and positive than −10 mV. Assuming that no saturation occurs, one expects a channel conductance of 1.26 nS in 0.3 M KCl in our conditions. This would be in agreement with the small conductance level found by Labarca et al. [7].

With a conductance (S) of 420 pS, and assuming the channel to be a hollow cylinder of length l filled with an aqueous solution of the same conductivity σ as the bulk solution and without specific binding site for ions, the effective pore diameter d ($= 2r$) of the channel can be estimated according to

$$r^2 = (S \cdot l)/(\sigma \cdot \pi)$$

with l as 6.8 nm [18] and σ in 100 mM KCl as 12 mS/cm [19]. The estimated diameter of the peroxisomal channel amounts to 1.7 nm. Labarca et al. [7] estimated a pore diameter of 1.5 and 3 nm. It should be stressed that these estimations are based on the assumption that permeation of ions occurs as free diffusion without interaction with binding sites in the channel protein.

Substates

For the peroxisomal channel in native membrane fragments at least eight substates could be distinguished. Conductance was linear in all cases. Substates were also found for the peroxisomal channel by Labarca et al. [7].

From a theoretical point of view two different types of mechanisms can be made responsible for the existence of substates [20]: the subunit type and the partial closure type. In the last type the channel consists of one single protein unit but the conductance can vary to different values because of conformational changes in the protein. In the subunit type each subunit can function as a conductance pathway, but more identical subunits can be organized in parallel and function as a large conductance channel if activated at the same time. The different substates are characterized by equally spaced current levels; the main conductance level is a multiple of the basic subunit level. Subunits however can also be organized in such a way that they aggregate like the staves of a barrel to form the walls of a single pore (the alamethicin type). Addition or deletion of subunits causes different substates. Such a behaviour is characterized by a stepwise increase or decrease in conductance, the amplitude of the change being dependent on the total number of subunits present at a given time.

The description of the subconductance behaviour for the peroxisomal channel allows us to exclude the subunit type. We therefore propose that the channel changes its conductance by a partial closure mechanism.

Kinetics

The closed probability of the peroxisomal channel in native membrane fragments is independent of the potential in contrast to the solubilized peroxisomal channel which displays a strong voltage-dependent closed probability. For native membrane fragments incorporated in planar lipid bilayers Labarca et al. [7] also found a strong voltage-dependent behaviour of the peroxisomal channel with a maximum around 0 mV, switching to apparently closed states at positive potentials and to partially closed states at negative voltages. The voltage-dependent behaviour of the channel remains obscure, and seems to depend either on the isolation procedure or method of reconstitution (planar lipid bilayer or patch). It is interesting to mention in this respect that the voltage-dependent gating of OmpF or OmpC porin channels that was found after reconstitution in planar lipid bilayers, was not detected in vivo in intact *Escherichia coli* cells [21]. This suggests that the voltage-dependent gating of those bacterial porins is an artifact of in vitro reconstitution.

Function, regulation, conclusion

The diameter of the peroxisomal channel as calculated above, is of the same magnitude as that of the mitochondrial porin [5]. It is therefore likely that the presence of this channel in the peroxisomal membrane explains the permeability of isolated peroxisomes to sucrose and to other small water-soluble molecules as well as the lack of latency of most peroxisomal enzymes when measured in broken cell systems [1–4,6]. Several questions remain unanswered, however. (1) Do amphiphilic compounds such as acyl-CoA esters, which are formed at the cytosolic side of the peroxisomal membrane [22] and which tend to partition into the lipid phase of the membrane, also penetrate via the hydrophilic channel or do they penetrate via a specific translocase? (2) is the permeability of the channel regulated in the intact cell? Regulation by a membrane potential is a possibility. The detergent-solubilized channel was voltage dependent, but the channel in its native membrane environment was not. Regulation by protein–protein interaction [23], protein phosphorylation, ions of other small effector molecules are other possibilities. The present experiments were performed in the presence of 1 mM Ca^{2+}. Omission of Ca^{2+} and addition of chelator did not influence the characteristics of behavior of the channel (unpublished results), making a regulatory role for Ca^{2+} unlikely.

The physiological role of the channel is probably to allow the rapid exchange of substrates, products and cofactors for peroxisomal enzymes between the peroxisomal and cytosolic compartments. However, the

intriguing possibility exists that the peroxisomal channel may also be part of the machinery responsible for the translocation of proteins across the peroxisomal membrane. As most mitochondrial proteins, peroxisomal matrix proteins are synthesized on free polyribosomes in the cytosol and posttranslationally imported in preexisting organelles [24]. It is believed that during translocation the imported proteins require unfolding to a translocation-competent conformation. In mitochondria, proteins are imported through an as yet unidentified hydrophilic membrane environment [25]. On theoretical grounds, Singer et al. [26] have proposed the involvement of a proteinaceous water-filled channel in the translocation of proteins across membranes. The diameter of the peroxisomal channel is sufficient to accommodate an unfolded protein. So, at least the theoretical possibility exists that the peroxisomal channel plays a role in protein translocation. A role in protein translocation for the peroxisomal channel does not exclude a concomitant role in the transport of small water-soluble molecules.

References

1 De Duve, C. and Baudhuin, P. (1966) Physiol. Rev. 46, 323–357.
2 Baudhuin, P. (1969) Ann. N.Y. Acad. Sci. 168, 214–228.
3 Van Veldhoven, P., Debeer L.J. and Mannaerts, G.P. (1983) Biochem. J. 210, 685–693.
4 Van Veldhoven, P.P., Just, W.W. and Mannaerts, G.P. (1987) J. Biol. Chem. 262, 4310–4318.
5 Benz, R. (1985) CRC Crit. Rev. Biochem. 19, 145–190.
6 Mannaerts, G.P. and Van Veldhoven, P.P. (1987) in Peroxisomes in Biology and Medicine (Fahimi, H.D. and Sies, H., eds.), pp. 169–176, Springer-Verlag, Berlin-Heidelberg.
7 Labarca, P., Wolff, D., Sato, U., Necochea, C. and Leighton, F. (1986) J. Membr. Biol. 94, 285–291.
8 Thieffry, M., Chich, J.-F., Goldschmidt, D. and Henry, J.-P. (1988) EMBO J. 7, 1449–1454.
9 Declercq, P.E., Haagsman, H.P., Van Veldhoven, P., Debeer, L.J., Van Golde, L.M.G. and Mannaerts, G.P. (1984) J. Biol. Chem. 259, 9064–9075.
10 Holloway, P.W. (1973) Anal. Biochem. 53, 304–308.
11 Tank, D.W. and Miller, C. (1983) Patch-Clamped Liposomes. in Single-channel recording (Sakmann, B. and Neher, E., eds.) pp. 91–105, Plenum Press, New York and London.
12 Coronado, R. and Latorre, R. (1983) Biophys. J. 43, 231–236.
13 Schlieper, P. and De Robertis, E. (1977) Arch. Biochem. Biophys. 181, 204–208.
14 Colombini, M. (1979) Nature 279, 643–645.
15 Roos, N., Benz, R. and Brdiczka, D. (1982) Biochim. Biophys. Acta 686, 204–214.
16 Fiek, C., Benz, R., Roos, N. and Brdiczka, D. (1982) Biochim. Biophys. Acta 688, 429–440.
17 Spray, D.C., Saez, J.C., Brosius, D., Bennett, M.V.L. and Hertzberg, E.L. (1986) Proc. Natl. Acad. Sci. USA 83, 5494–5497.
18 Fujiki, Y., Shio, H., Hubbard, A.L. and Lazarow, P.B. (1982) J. Cell Biol. 93, 103–110
19 Fox, J.A. (1987) J. Membr. Biol. 97, 1–8.
20 Benz, R., Darveau, R.P. and Hancock, R.E.W. (1984) Eur. J. Biochem. 140, 319–324.
21 Sen, K., Hellman, J. and Nikaido, H. (1988) J. Biol. Chem. 263, 1182–1187.
22 Mannaerts, G.P., Van Veldhoven, P.P., Van Broekhoeven, A., Vandenbroek, G. and Debeer, L.J. (1982) Biochem. J. 204, 17–23.
23 Holden, M.C. and Colombini, M. (1988) FEBS Lett. 241, 105–109.
24 Lazarow, P.B. and Fujiki, Y. (1985) Annu. Rev. Cell Biol. 1, 489–530.
25 Pfanner, N., Hartl, F.-U., Guiard, B. and Neupert, W. (1987) Eur. J. Biochem. 169, 289–293.
26 Singer, S.J., Mahen, P.A. and Yaffe, M.P. (1987) Proc. Natl. Acad. Sci. USA 84, 1015–1019.