Evidence for the Presence of a Porin in the Membrane of Glyoxysomes of Castor Bean

Sigrun Reumann, Maren Bettermann, Roland Benz, and Hans W. Heldt*


Glyoxysomes of endosperm tissue of castor bean (Ricinus communis L.) seedlings were solubilized in a detergent and added to a lipid bilayer. Conductivity measurements revealed that the glyoxysomal preparation contained a porin-like channel. Using an electrophysiological method, which we established for semiquantitative determination of porin activity, we were able to demonstrate that glyoxysomal membranes purified by sucrose density gradient centrifugation contain an integral membrane protein with porin activity. The porin of glyoxysomes was shown to have a relatively small single-channel conductance of about 330 picosiemens in 1 M KCl and to be strongly anion selective. Thus, the glyoxysomal porin differs from the other previously characterized porins in the outer membrane of mitochondria or plastids, but is similar to the porin of spinach (Spinacia oleracea L.) leaf peroxisomes. Our results suggest that, in analogy to the porin of leaf peroxisomes, the glyoxysomal porin facilitates the passage of small metabolites, such as succinate, citrate, malate, and aspartate, through the membrane.

Peroxisomes are organelles enclosed by a single membrane. They are found in almost all eukaryotic cells (Cavalier-Smith, 1987) and it is typical for them to have at least one H$_2$O$_2$-forming oxidase and CAT, which degrades H$_2$O$_2$ (de Duve and Baudhuin, 1966). The main types of plant peroxisomes are leaf peroxisomes, in which enzymes of glycolate metabolism are compartmentalized (Tolbert, 1980), and glyoxysomes, which are involved in the metabolism of fatty acids (Breitenbach and Beevers, 1967). Glyoxysomes have been characterized most extensively in oilseed plants, e.g. castor bean (Ricinus communis L.) during germination, where they are involved in mobilizing storage lipids to provide nutrients for growing seedlings.

Several metabolites have to pass the glyoxysomal membrane during metabolism. Glyoxysomes take up fatty acids, convert them by β-oxidation to acetyl-CoA, and then further via the glyoxylate cycle to succinate, which is released from the glyoxysomes as a precursor of gluconeogenesis. Recent results suggest that a cytosolic aconitase is involved in the glyoxylate cycle (Courtois-Verniquet and Douce, 1993; Debellis et al., 1994; Hayashi et al., 1995), which implies that citrate and isocitrate have to pass through the glyoxysomal membrane. Mettler and Beevers (1980) concluded from their results that a malate-aspartate shuttle operates in oxidizing the NADH formed by β-oxidation of fatty acids and by the glyoxylate cycle.

Membrane proteins involved in the transport of metabolites have not yet been identified in glyoxysomes. The results of permeability studies of peroxisomes of different origin are contradictory (Mettler and Beevers, 1980; Donaldson et al., 1981; Verleur and Wanders, 1993; Van Roermund et al., 1995). Mettler and Beevers (1980) found that various metabolites traversed the glyoxysomal membrane by unspecific diffusion, but discussed the possibility that their experimental manipulations may have damaged the fragile glyoxysomal membrane and so prevented the detection of specific translocators.

Although glyoxysomes and leaf peroxisomes are specialized for different metabolic functions, small metabolites, mainly organic acids and amino acids, have to pass the peroxisomal membrane in both types of peroxisomes. Recently, we provided evidence that the membrane of leaf peroxisomes of spinach (Spinacia oleracea L.) contains a porin. The porin is characterized by a relatively low single-channel conductance (350 pS in 1 M KCl), a strong anion selectivity (Reumann et al., 1995), a voltage dependence of conductance at low electrolyte concentration, and a specific dicarboxylate-binding site (Reumann et al., 1996; S. Reumann, E. Maier, H.W. Heldt, and R. Benz, unpublished results). We have shown that the porin is permeable to glycolate, glycerate (Reumann et al., 1995), and several dicarboxylates, such as malate, oxaloacetate, α-ketoglutarate, and glutamate (S. Reumann, E. Maier, H.W. Heldt, and R. Benz, unpublished results), which have to pass the peroxisomal membrane during photosynthetic metabolism.

Porin-like channels have been reported to occur in the membranes of peroxisomes from animals (van Veldhoven et al., 1987) and yeast (Sulter et al., 1993), but with properties similar to the mitochondrial porin (Labarca et al., 1986; Lemmens et al., 1989; Kaldi et al., 1993; Sulter et al., 1993). The mitochondrial porin, as well as the porins of the outer membrane of chloroplasts (Flügge and Benz, 1984)

Abbreviations: CAT, catalase; MS, malate synthase; nS, nanosiemens; pS, picosiemens.
and amyloplasts (Fischer et al., 1994a), form large general diffusion channels like those in the outer membrane of Gram-negative eubacteria (Benz, 1994a). According to our present knowledge these diffusion channels are formed by amphipathic $\beta$-sheets arranged in a barrel-like structure (Benz, 1994a, 1994b).

This report shows that the glyoxysomal membrane contains a pore-forming protein, referred to as porin, which allows the diffusion of metabolites across the glyoxysomal boundary membrane, and its properties will be described.

MATERIALS AND METHODS

Plant Material and Isolation of Glyoxysomes

Seeds of castor bean (Ricinus communis L., “Zanzibarensis Gemisch”) were surface-sterilized (0.075% [w/v] CaCl$_2$O$_4$) and soaked overnight in water prior to germination at about 30°C in the dark for 5 d. According to Vigil et al. (1987), glyoxysomes were isolated from the endosperm tissue with the modification that, in addition to homogenizing the endosperm with razor blades, a polytron homogenizer was used. The glyoxysomal fraction of the Suc-density gradient was taken from 44.0 to 57.8% (w/w) Suc. For determination of the activity of membrane-bound Cyt c-reductase about 70% of the gradient fraction was slowly diluted to a Suc concentration of 0.5 M, and the membranes were sedimented by ultracentrifugation (for 1 h at 100,000g, Kontron TFT 45.94, Zurich, Switzerland).

Isolation of Glyoxysomal Membranes

Glyoxysomes were slowly diluted with buffer (10 mM Hepes, pH 7.5, 0.8 mM MgCl$_2$) to about 0.5 m Suc and centrifuged (for 1 h at 140,000g, Kontron TFT 65.13). The sedimented membranes and adhering matrix proteins were resuspended in buffer, and the suspension was subjected to 10 freeze/thaw cycles (freezing in liquid nitrogen, thawing at room temperature) followed by a thorough homogenization in a Potter homogenizer. The suspension (0.8 mL, containing about 2 mg of protein) was loaded onto a linear Suc gradient (1.13–1.26 g/mL, 10 mM Hepes, pH 7.5, and 0.8 mM MgCl$_2$, in 4.0 mL volumes) and centrifuged in a swing-out rotor (for 15 h at 240,000g, AH 650, Sorvall). The gradient was fractionated from the top (fraction 1, 1.0 mL; fractions 2–10, 0.4 mL). The fractions for enzyme activities were diluted 1:2 in 10 mM Hepes, pH 7.5, and stored at –80°C. Samples for the determination of porin activity were adjusted to a detergent concentration of 5 mg/mL Genapol by a 1:2-fold dilution and were frozen in small aliquots.

Isolation of Membranes of Castor Bean Proplastids and Spinach (Spinacia oleracea L.) Chloroplasts

Castor bean endosperm proplastids were isolated according to Milfin and Beevers (1974). The organelles were disrupted osmotically by dilution with 10 mM Hepes, pH 7.5, to about 0.5 m Suc and incubated for 15 min on ice, and the membranes were then sedimented (for 1 h at 100,000g, 65.13, Kontron TFT). Spinach chloroplasts were isolated according to Heldt and Sauer (1971), and chloroplast envelope membranes were isolated according to Douce et al. (1973).

Measurement of Enzyme Activities

If not stated otherwise, measurements of marker enzyme activities were carried out at 25°C with a detergent concentration of 0.1% (v/v) Triton X-100. CAT was measured as described previously (final volume, 1 mL) (CAT; Stegink et al., 1987). The measurement of NADH-Cyt c reductase (600 µL) (Sauer and Robinson, 1985), Cyt c oxidase (700 µL) (0.02% [v/v] Triton X-100) (Douce et al., 1973), MS (1 mL) (Miernyk et al., 1979), and shikimate oxidoreductase (600 µL) (Fiedler and Schulz, 1985) was performed as previously described. Protein was determined according to Peterson (1977).

SDS-PAGE and Western-Blot Analysis

Precipitation of protein fractions was carried out using chloroform/methanol (Wessel and Flügge, 1984). SDS-PAGE was performed on 12.5% gels according to Laemmli (1970). Polypeptide bands were detected by silver-staining (Blum et al., 1987). In western-blots analysis the bound antibodies were detected by a peroxidase-coupled second antibody using an enhanced chemoluminescence kit (Amersham-Buchler, Braunschweig, Germany).

Lipid Bilayer Experiments

The methods used for the bilayer experiments have been described in detail elsewhere (Benz et al., 1987; Schmid et al., 1992). Glyoxysomal membranes were solubilized in 0.5% Genapol X-80 (Fluka) and added in a concentration of about 1 µg/mL protein to the aqueous-phase bathing lipid-bilayer membranes from diphytanoyl-phosphatidylcholine/n-decane (Avanti Polar Lipids, Alabaster, AL). Determination of porin activity was performed by measuring the membrane current at a constant voltage.

RESULTS

Isolation of Glyoxysomes and Purification of Glyoxysomal Membranes

Planar lipid-bilayer experiments of the sensitivity required to resolve the low single-channel conductance of the peroxisomal porin and to quantify porin activity can only be performed with highly purified organelle preparations. To identify the origin of the putative glyoxysomal porin we analyzed the contamination of the glyoxysomal fraction. After the enrichment of glyoxysomal membranes by Suc-density gradient centrifugation, the relationship between the content of glyoxysomal membranes and of porin activity in these purified fractions was evaluated.
From measuring the activities of CAT and MS as peroxisomal marker enzymes, the yield of the glyoxysomes obtained from endosperm of castor bean was evaluated as 6 and 12%, respectively (Table I). The contamination of the glyoxysomal preparation with ER membranes, mitochondria, and proplastids was low, as the recoveries of the corresponding marker enzyme activities were 2, 1, and 0.8%, respectively, as compared with the starting homogenate (Table I). The relatively high recovery of Cyt c reductase in the purified glyoxysomal fraction reflects the localization of an isoenzyme in the glyoxysomal membrane (Hicks and Donaldson, 1982). From the lower recovery of CAT, the contamination of the glyoxysomal fraction with mitochondria and proplastids is evaluated as 16 and 12%, respectively.

Sodium carbonate treatment (pH 11.5) to solubilize the matrix proteins adhering to the peroxisomal membrane (Fujiki et al., 1982) led to a complete inactivation of porin activity of spinach leaf peroxisomes (Reumann et al., 1995) and castor bean glyoxysomes (data not shown). Therefore, glyoxysomal membranes were purified by subjecting glyoxysomes to an osmotic shock, followed by sedimentation of the membranes and Suc-density gradient centrifugation. The osmotic shock resulted in the solubilization of about 60% of the organelle protein (data not shown). The adhesion of matrix proteins to the glyoxysomal membrane was kept at a minimum by thorough homogenization (see “Materials and Methods”). As shown in Figure 1, a further separation of matrix enzymes and membrane proteins was achieved by Suc-density gradient centrifugation. Some of the glyoxysomal matrix proteins appeared in the upper part of the gradient (fractions 2–4; \( \rho = 1.12-1.17 \) g/mL; Fig. 1, A and B) at a density similar to that of matrix proteins of spinach leaf peroxisomes (Reumann et al., 1995). The main activity of MS, which tends to stick to the glyoxysomal membrane (Chapman et al., 1989), was found in fractions 5 to 7 at a density of about 1.19 g/mL, indicating that most of the glyoxysomal membranes were contained in these fractions. The density of the membrane was slightly lower than that of membranes treated with sodium carbonate (\( \rho = 1.22 \) g/mL; data not shown).

Probably because of the close cellular association of peroxisomes with plastids and mitochondria (Huang et al., 1983), fractions of peroxisomal membranes tend to be contaminated with the outer membranes of these organelles. Because the outer membranes of mitochondria (Schmid et al., 1992; Aljamal et al., 1993) and plastids (Flügge and Benz, 1984; Fischer et al., 1994a) are known to contain porins, we checked their contamination by electrophysiological and immunological means. A contamination of the glyoxysomal membrane fraction with outer membranes of mitochondria could be excluded by electrophysiological measurements, since no mitochondrial porin activity with a single-channel conductance in the range of 2 or 4 nS in 1 m KCl was observed (see Fig. 5, A and B).

Polyclonal antibodies raised against the 24-kD protein of the outer envelope membrane of spinach chloroplasts (Fischer et al., 1994b) were used to investigate whether castor bean proplastids contain a homologous membrane protein that could be used as a marker protein. Proplastids, the density of which is similar to that of glyoxysomes, were separated from glyoxysomes by rate-zonal centrifugation. An aliquot of each fraction was subjected to an osmotic shock and the membranes were sedimented by ultracentrifugation. Polyclonal antibodies raised against the 24-kD protein of the outer envelope membrane of spinach chloroplasts (Fischer et al., 1994b) showed a cross-reaction with the blotted proteins, the intensity of which correlated with the content of proplastids (Fig. 2, B and C). This result led to the conclusion that a homologous protein is located in the outer membrane of proplastids of castor bean. Western-blot analysis performed with the glyoxysomal membrane fraction (Fig. 1) revealed no such cross-reaction (Fig. 3), indicating that there was only slight contamination with outer envelope membranes of proplastids. The cross-reactivity with a protein of a higher molecular weight was judged as an unspecific artifact.

### Table 1. Activities and recoveries of marker enzymes in the suspension of glyoxysomes

The contamination of the glyoxysomal preparation by other organelles was evaluated. The recovery is related to the total activity in the crude extract after homogenization of castor bean endosperm (mean values ± SD of data from four independent preparations; Cyt c reductase n = 3). The enzyme activities were measured directly in the crude extract and in the gradient fraction with the exception of the membrane enzyme Cyt c reductase, the activity of which was determined in the membrane sediment after ultracentrifugation.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific Activity in the Crude Extract</th>
<th>Specific Activity in the Glyoxysomal Suspension</th>
<th>Total Activity in the Glyoxysomal Suspension</th>
<th>Enrichment</th>
<th>Recovery of Total Activity in the Glyoxysomal Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol (mg protein)}^{-1} \text{ min}^{-1} )</td>
<td>( \mu \text{mol min}^{-1} )</td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Glyoxysomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0.13 ± 0.02</td>
<td>1.34 ± 0.19</td>
<td>5.51 ± 1.64</td>
<td>10.2</td>
<td>12.3</td>
</tr>
<tr>
<td>Catalase</td>
<td>401 ± 116</td>
<td>2220 ± 910</td>
<td>8510 ± 1820</td>
<td>5.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Proplastids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shikimate oxidoreductase</td>
<td>(5.66 ± 0.26) × 10^{-3}</td>
<td>(3.57 ± 1.29) × 10^{-3}</td>
<td>(14.7 ± 7.2) × 10^{-3}</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>(280 ± 51) × 10^{-3}</td>
<td>(245 ± 140) × 10^{-3}</td>
<td>(921 ± 350) × 10^{-3}</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Cyt c oxidase</td>
<td>(2.19 ± 0.18) × 10^{-3}</td>
<td>(1.27 ± 0.29) × 10^{-3}</td>
<td>(17.6 ± 3.8) × 10^{-3}</td>
<td>0.6</td>
<td>2.0</td>
</tr>
</tbody>
</table>

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Identification of a Porin in the Membrane of Glyoxysomes

Enriched glyoxysomal membranes or glyoxysomal membranes purified by Suc-density gradient centrifugation were treated with the nonionic detergent Genapol X-80 (final concentration 0.5%) to solubilize the membrane proteins. The detergent extract was added in a concentration of about 1 μg/mL glyoxysomal membrane protein to the aqueous-phase bathing a lipid-bilayer membrane. The membrane current was measured at a fixed voltage of 10 mV. After a lag time of about 3 to 4 min, presumably caused by slow aqueous diffusion of the protein, the membrane current started to increase in a stepwise fashion (see Fig. 4 for an experiment with glyoxysomal membranes). Each conductance step shown in this figure corresponded to the incorporation of a single-channel-forming unit into the membrane. The average single-channel conductance of several experiments was about 330 pS in 1 M KCl (see Fig. 5, A and B).

To provide conclusive evidence for the localization of the porin, we developed a new procedure to assay semiquantitatively the amount of native porin molecules in a sample, which was carried out as follows: Small quantities (20 μL) of the fractions dissolved in Genapol X-80 were added to the aqueous phase on both sides of the artificial bilayer (final protein concentration in the aqueous phase 0.06 μg/mL) and the electrolyte solution was stirred to allow equilibration. After a short time lag, probably caused by diffusion of the solubilized protein molecules through the aqueous phase and by oligomerization of monomers, the conductance of the membrane began to increase (Fig. 6A). The time course was similar to that described previously for porins of mitochondrial or bacterial origin (Benz et al., 1980; Roos et al., 1982). We decreased the sensitivity of single-channel recording by a factor of 50, so that the single-conductance steps were not resolved anymore. We determined the continuous time-dependent increase of the macroscopic membrane conductance as a measure of the rate of porin reconstitution in the bilayer membrane, and hence as a measure of porin concentration in the bathing medium. The rate of porin reconstitution increased for about 5 min before reaching a maximal value (Fig. 6B).
Several bilayer membranes had to be prepared to carry out measurements in which the rate of reconstitution was not limited by a burst of the bilayer membrane during the experiment. The average maximal rate of porin reconstitution of at least three membranes correlated positively with the amount of peroxisomal protein added (Fig. 7). As the measurements were carried out with a constant sample volume and under identical conditions, the measurement of the maximal rate of porin reconstitution could be taken as a semiquantitative measure for the peroxisomal porin activity in the sample.

As shown in Figure 1C, fractions 5 and 6 contained the highest porin activity of about 600 nS/min, which is equal to about 30 reconstitution events per second per 15 μg of protein of a porin with a single-channel conductance of about 350 pS. The porin activity correlated with the activity of the membrane-associated MS (Fig. 1, B and C). Control experiments confirmed that the addition of the detergent Genapol X-80, at a concentration range used for the solubilization of the protein, led to no appreciable increase in the membrane conductance. In the fractions where peroxisomal matrix enzymes were found (fractions 2–4), only low-porin activity was determined. These data provide conclusive evidence that the membrane of glyoxysomes contains a porin forming a channel with a single-channel conductance of about 330 pS in 1 M KCl.

Finally, we analyzed the effect of high ionic strength on the solubilization of porin activity. Peroxisomal membranes were incubated for 15 minutes in a solution containing 300 mM NaCl and 150 mM KCl before being subjected to ultracentrifugation. The resulting membrane sediment contained about 40% of total proteins. The glyoxysomal porin activity remained associated with the membrane during this procedure, indicating that the porin represents an integral membrane protein (data not shown).

Electrophysiological Properties of the Glyoxysomal Porin

The channel formed by the glyoxysomal porin was permeable for a variety of ions (Table II). The average single-channel conductance in 1 M KCl (340 pS) was the same as in 1 M LiCl (340 pS), but it was considerably smaller in 1 M potassium acetate (30 pS). Because K⁺, Cl⁻, Li⁺, and acetate have about the same aqueous mobility (Castellan, 1983), these results indicate that the single-channel conductance is mainly dependent on the nature of the anion and that the channel has a preference for anions. From several experiments we determined the ratio of cation permeability to anion permeability p_c/p_a of about 0.05 ± 0.02 (n = 10, data not shown) for KCl, which demonstrates that with this electrolyte the permeability of anions is at least 20 times higher than that of cations.

Succinate is a major metabolite of glyoxysomal metabolism. To study the physiological function of the porin in glyoxysomal metabolism, we performed planar bilayer experiments in a solution of potassium succinate, yielding an average single-channel conductance of 30 pS (Table II). This result shows that succinate permeates this channel.

To analyze the relationship between the single-channel conductances (G) and the KCl concentrations, these have to be divided by the corresponding specific activities of the electrolyte (σ). Note that especially at higher concentrations the specific electrolyte activity is not proportional to the KCl concentration. The data of Table II show that with increasing KCl concentration the G/σ ratio decreases. The conductance of the pore cannot be explained in terms of a simple diffusion through an aqueous pore, indicating that...
the glyoxysomal porin contains a binding site for anions, as found earlier with the porin of leaf peroxisomes (Reumann et al., 1996; S. Reumann, E. Maier, H.W. Heldt, and R. Benz, unpublished results). The porins of plastids and mitochondria are voltage-regulated at low transmembrane potentials (Flügge and Benz, 1984; Benz, 1994b; Fischer et al., 1994a). The single-channel conductance of the porin of the glyoxysomes, however, is dependent on the voltage only at a low electrolyte concentration of 0.2 M KCl (Fig. 8), whereas at high KCl concentrations of 1 M KCl the single-channel conductance is not affected by a transmembrane potential as high as 50 mV (data not shown).

**DISCUSSION**

Our results demonstrate that the membrane of glyoxysomes of castor bean contains a porin, as concluded from the following results: (a) The isolated glyoxysomes used for these studies were only contaminated to a minor extent by mitochondria and proplastids (about 16 and 12%, respectively), as determined by the measurement of marker enzyme activities. (b) The analysis of subfractionated glyoxysomes showed a correlation between porin activity and the concentration of glyoxysomal membranes in the gradient fractions. (c) The porin activity was shown to be associated with an integral membrane protein of glyoxysomes. (d) Immunological and electrophysiological analysis showed that the fractions containing enriched glyoxysomal membranes were not markedly contaminated by outer membranes of plastids or mitochondria. (e) In regard to single-channel conductance, ion selectivity, and voltage dependence, the investigated glyoxysomal porin was found to be distinctly different from the porins contained in the outer membrane of mitochondria and plastids.

**Figure 5.** Histogram of the probability of the occurrence of certain conductivity units observed with membranes formed from diphytanoyl phosphatidylcholine/n-decane in the presence of detergent-solubilized enriched glyoxysomal membranes (A) and glyoxysomal membranes purified further by Suc-density gradient centrifugation (B). The aqueous phase contained 1 M KCl (pH 6.0) and 0.26 μg/mL (A) and 1.1 μg/mL protein (B). The applied membrane potential was 10 mV and the temperature was 25°C. The average single-channel conductance was 340 (A) and 320 (B) for 292 (A) and 156 (B) single-channel events. In A one single-channel event of 7.3 nS was observed and not counted for determination of the average single-channel conductance. P(G), Probability of the single-channel conductance G.

**Figure 6.** Time course of the reconstitution of the porin of glyoxysomes into membranes formed of diphytanoyl phosphatidylcholine/n-decane. Increase of the membrane current (A) shown after the addition of Genapol-solubilized integral membrane proteins of glyoxysomes to a black lipid-bilayer membrane as a function of time and the rate of porin incorporation as a function of time (B). The arrows indicate the time points where the pen of the chart was reset to allow further measurement. The aqueous phase contained about 0.06 μg protein/mL, 17 μg Genapol/mL, and 1 M KCl. The membrane was formed from diphytanoyl phosphatidylcholine/n-decane. The voltage applied was 10 mV and the temperature was 25°C.
Porin of Glyoxysomes

Our results indicate that the electrophysiological properties of glyoxysomal porin are almost identical to those of the porin of spinach leaf peroxisomes (Reumann et al., 1995; S. Reumann, E. Maier, H.W. Heldt, R. Benz, unpublished results). The average single-channel conductances in various concentrations of KCl and the voltage dependence are similar. Like the porin of leaf peroxisomes, the glyoxysomal porin is strongly anion-selective. The nonlinear relationship between the salt concentration and the single-channel conductance of the glyoxysomal porin indicates that both porins contain a specific binding site for certain anions. In contrast, the porins of the outer membrane of mitochondria and plastids are characterized by a 10- to 20-fold higher single-channel conductance (mitochondria, 2–4 nS; Schmid et al., 1992; Aljamal et al., 1993; nongreen plastids, 2–4 nS, Fischer et al., 1994a; and chloroplasts, 7–8 nS, Flügge and Benz, 1984) and a strong voltage dependence in 1 M KCl. And as typical for general diffusion pores, these porins show a linear relationship between the single-channel conductance and the concentration of potassium chloride. Their anion selectivity is much weaker than that of the peroxisomal porins.

Unlike other known eukaryotic porins, the porins of leaf peroxisomes and glyoxysomes do not form a large general diffusion pore, as for instance, the porins of the outer membranes of mitochondria and plastids (Flügge and Benz, 1984; Benz, 1994b). The porin of spinach leaf peroxisomes contains a binding site specific for dicarboxylic acids (Reumann et al., 1996), which increases the permeability of the porin for these substrates. With these specific properties the porin of leaf peroxisomes is unique among the porins of eukaryotic cells and resembles some prokaryotic porins that are induced under specific growth conditions, e.g., LamB of Escherichia coli for transport of sugars, Tsx of E. coli for transport of nucleosides, and OprP of Pseudomonas aeruginosa for phosphate transport (Hancock et al., 1982; Benz and Hancock, 1987; Benz et al., 1987; Maier et al., 1988). These prokaryotic porins and the porin of leaf peroxisomes have a narrow diameter in common (about 0.6 nm for the leaf peroxisomal porin; S. Reumann, E. Maier, H.W. Heldt, and R. Benz, unpublished results) and a binding site for specific substrates inside the channel.

The porin of spinach leaf peroxisomes, with its specific binding site for certain anions, enables the passage of photosynthetic metabolites, such as glycolate, glycерate, malate, oxaloacetate, α-ketoglutarate, and glutamate (Reumann et al., 1995; S. Reumann, E. Maier, H.W. Heldt, and R. Benz, unpublished results). Because of the similar properties, we predict that the glyoxysomal porin makes the glyoxysomal membrane permeable to metabolites of glyoxysomal metabolism, such as succinate, malate, aspartate, α-ketoglutarate, glutamate, citrate, and isocitrate. A permeability of the glyoxysomal porin to potassium succinate has been demonstrated in this study.

The presence of a porin in an organelle that is surrounded by a single membrane raises the question of how

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**Table II. Average single-channel conductance (G) of glyoxysomal porin in different salt solutions**

From the specific activity of the electrolyte (α) (Benz, 1994b) the G/α-ratio was evaluated. The membranes were formed of diphytanoyl phosphatidylcholine dissolved in n-decane. The aqueous solutions were unbuffered and had a pH of 6.0 unless otherwise indicated. For K-succinate, 1 M succinic acid was adjusted with KOH to pH 6.5. The applied voltage was 10 mV and the temperature was about 25°C. The average single-channel conductance was calculated from at least 100 single events.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration mol L⁻¹</th>
<th>G α</th>
<th>G/α 10⁻¹⁰ m</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>0.3</td>
<td>190</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>260</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>340</td>
<td>112</td>
</tr>
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<td></td>
<td>3.0</td>
<td>510</td>
<td>280</td>
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<td>LiCl</td>
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<td>340</td>
<td>71</td>
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<tr>
<td>K-acetate</td>
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</tr>
<tr>
<td>K-succinate</td>
<td>1.0</td>
<td>30</td>
<td>—</td>
</tr>
</tbody>
</table>

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**Figure 7. Maximal rate of the incorporation of glyoxysomal porin into membranes formed of diphytanoyl phosphatidylcholine/n-decane as a function of protein content.**

**Figure 8. Voltage dependence of the porin of glyoxysomes in 0.2 M KCl. The ratio of the conductance, G, at a given voltage, V, is divided by the conductance G₀, at 10 mV as a function of the voltage.**
compartimentation of metabolism is accomplished. In leaf peroxisomes the compartmentation of metabolism is not due to the permeability properties of the boundary membrane, but is caused by the peroxisomal matrix proteins acting as a multi-enzyme complex, allowing metabolite channeling and thus preventing metabolic intermediates from leaking into the cytosol (Heupel and Heldt, 1994). There are also indications for multi-enzyme-like structures in glyoxysomes, where a specific arrangement of isocitrate lyase and MS (Beeckmans et al., 1994) and of malate dehydrogenase and MS (Guex et al., 1995) has been demonstrated.

The characterization of an almost identical transport protein in the two functionally different types of plant peroxisomes, leaf peroxisomes and glyoxysomes, raises the question of whether membranes of plant peroxisomes and of nonplant peroxisomes contain a homologous porin. Plant glyoxysomes, mammalian peroxisomes, and glyoxysome-like peroxisomes in fungi and algae carry out β-oxidation of fatty acids. On the other hand, as mentioned in the introduction, peroxisomes from yeast and liver may contain pores with properties similar to those of the outer membrane of mitochondria (Labarca et al., 1986; van Veldhoven et al., 1987; Lemmens et al., 1989; Sultner et al., 1993) in forming a large, unspecific diffusion pore, e.g. in mitochondria with a diameter of about 1.7 nm. In general, unspecific diffusion pores of the outer membrane of Gram-negative bacteria (Benz, 1994a), mitochondria (Benz, 1994b), and plastids (Flügge and Benz, 1984; Fischer et al., 1994a) are localized in an outer membrane of a compartment surrounded by two membranes, where they give this membrane sieve-like properties, whereas the inner membrane represents the selective permeability barrier. The comparatively small aperture of the peroxisomal pores and their anion specificity may control the flux of metabolites across the boundary membrane and thus participate in the compartmentation of peroxisomal metabolism.

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