Metabolite transport across the peroxisomal membrane

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Peroxisomes are single-membrane bound organelles that are present in virtually all eucaryotic cells. They are involved in numerous important metabolic pathways. Mammalian peroxisomes are currently known to perform a role in fatty acid alpha- and beta-oxidation, the degradation of purines, polyamines, L-pippecolic acid and D-amino acids, and the biosynthesis of etherphospholipids and bile acids. The peroxisomal localization of these functions raises the question of how the transport across the peroxisomal membrane is accomplished. Soon after the discovery of the organelle, it was observed that the membrane of purified, isolated peroxisomes is freely permeable to most metabolites. More recently, however, considerable evidence was gained that strongly suggests that, at least in vivo, the peroxisomal membrane constitutes a permeability barrier to several metabolites, including NADP(H), NAD(H), and acetyl-CoA. These findings imply the presence of transporters in the peroxisomal membrane to support peroxisomal metabolism.

The presence of glutathione-utilizing enzymes in peroxisomes of various species has been reported. In light of the impermeability of the peroxisomal membrane in vivo, this raises the question of how glutathione is regenerated. As described in chapter 2, peroxisomes of the yeast Saccharomyces cerevisiae were found to contain glutathione reductase. This enzyme probably serves to maintain a supply of reduced glutathione in the peroxisomal compartment, using NADPH as a source of reducing potential. Interestingly, it was found that the targeting of Grl1p proceeds in an unusual fashion, since disrupting either of the two known protein import receptors for peroxisomal matrix proteins (pex5p and pex7p) had no effect on the peroxisomal localization of Grl1p.

In recent years, a number of peroxisomal metabolite transporters have been identified. Firstly, the mammalian peroxisomal membrane contains four members of the superfamily of ABC-transporters, called ALDP, ALDR, PMP70 and PMP69. Mutations in the ALDP protein are the cause for the severe human disorder X-linked adrenoleukodystrophy. It is currently believed that the protein is involved in the import of acyl-CoA units into the peroxisomes. The function of the other peroxisomal ABC-transporters remains unknown.

In addition, peroxisomes contain a member of the Mitochondrial Carrier Family (MCF / SLC25) of solute transporters, called Ant1p or PMP34 (in S. cerevisiae and man respectively). This protein catalyzes the exchange of adenine nucleotides across the peroxisomal membrane, which was demonstrated directly by functional reconstitution of the purified protein in liposomes, thereby circumventing the experimental problems associated with the high permeability of the membrane of isolated peroxisomes. Our results with the human protein PMP34 are described in Chapter 3. The adenine nucleotide transporter presumably serves to supply intraperoxisomal acyl-CoA synthetases with ATP. This enzyme, however, also yields pyrophosphate as one of its products, and it is therefore conceivable that this compound, or orthophosphate resulting from its hydrolysis, is exported from the peroxisomes. This prompted us to investigate the presence of a pyrophosphate transporter in the peroxisomal membrane, using a similar approach as employed for the measurements of PMP34 activity. As described in chapter 4, we found that mammalian peroxisomal membranes contain a protein that confers permeability to phosphate and pyrophosphate upon reconstitution in proteoliposomes. The protein differs from the mitochondrial phosphate transporter by several properties, including its sensitivity to inhibitors and mechanism of transport. We speculate that this transporter may be required to support the activity of intraperoxisomal acyl-CoA synthetase.

It was proposed previously that the transport of redox equivalents across the peroxisomal membrane occurs in the form of mono-, di- and tricarboxylates, including isocitrate and 2-ketogluatrate (for the regeneration of NADPH), malate and oxaloacetate (for NAD+ regeneration in S. cerevisiae).
and possibly also pyruvate and lactate (for NAD⁺ regeneration in mammals). To detect the possible presence of transporters for these metabolites, the ability of proteoliposomes containing peroxisomal membrane protein to take up various radiolabeled substrates was investigated, as described in chapter 5. It was observed that the peroxisomal membrane contains one or more proteins that facilitate the translocation of 2-ketoglutarate and isocitrate. It was also shown that proteoliposomes containing peroxisomal protein do not become permeable to NADPH and NADH, in line with the finding that the peroxisomal membrane is impermeable to these compounds. Furthermore, it was found that the translocation of pyruvate and lactate in a protonated form proceeds readily even without the aid of transporters. Although this prevented us from detecting the possible presence of proton-linked monocarboxylate transporters previously proposed to exist in the peroxisomal membrane, this finding may imply that a transporter may not be necessary for the translocation of these compounds, depending on the organelle and cytosolic pH and the metabolic requirements of peroxisomal metabolism. In addition, it suggests that caution must be observed in the interpretation of experiments regarding *in vitro* measurements of transporters for these compounds.

In light of the combined results, from this work and previously reported by others, the presence of a large, non-specific channel in the peroxisomal membrane, as was suggested in the past, appears unlikely. However, the possibility remains that the peroxisomal membrane is permeable to a wide range of substrates, but not NADP(H), NAD(H), CoA-derivate and perhaps several other compounds. Given the earlier findings that both (pyro)phosphate and 2-ketoglutarate may be transported into proteoliposomes in unidirectional manner, we anticipated that the permeability towards various compounds could also be detected by observing the osmotic behaviour of proteoliposomes containing reconstituted peroxisomal membrane protein. This method has been effective in the detection and characterization of many transporters and channels in the past. As described in chapter 6, reconstitution of peroxisomal membrane protein into proteoliposomes indeed does render the liposome membrane permeable to a number of small metabolites involved in peroxisomal metabolism. Interestingly, the membrane remains impermeable to NADP(H), NAD(H), AMP, oxidized glutathione and several other compounds with a relatively large molecular weight, in agreement with the permeability of the peroxisomal membrane *in vivo*.

In the past, the ability to functionally reconstitute transporters has proven to be a very useful tool for purification and identification of unknown transporters in various intracellular membranes. The availability of the transport assays described in this thesis now makes it possible to identify and further characterize unknown transporters in the peroxisomal membrane.