

## Biosynthesis of Peroxisomal Proteins\*

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The proteins of the different intracellular membranes are synthesized both on bound and free ribosomes.<sup>1</sup> In most cases, however, it is not known to what extent the individual ribosomal compartments contribute for peroxisomes. It is known that catalase is synthesized on free ribosomes and transferred directly to peroxisomes without hydrolytic processing of the polypeptide chain.<sup>2</sup> Morphological investigations suggested that peroxisomes originate from the endoplasmic membrane system as an outgrowth but the peptide patterns on slab gel differ from those of the microsomes.<sup>3</sup> Naturally the transport of proteins could be selective and involve only a few proteins; consequently the peptide pattern is not necessarily identical in the two membranes. The purpose of this investigation was to find out whether peroxisomal precursor peptides were present in the microsomal fraction or in the supernatant after *in vivo* labeling.

In order to identify peroxisomal peptides in the various fractions, peroxisomes were prepared and soluble proteins (content) were removed by treatment of the fraction with a low concentration of deoxycholate. The membranes were solubilized with a high concentration of detergent and the particulate urate oxidase core was removed by centrifugation. The supernatant containing exclusively peroxisomal membrane proteins was used to prepare antibodies against all proteins of the peroxisomal membranes.

The antibodies prepared against proteins of the peroxisomal membranes could be used to follow the biosynthesis and transport of these proteins. Rats were injected *via* the portal vein with an amino acid mixture of high specific radioactivity. Microsomes, peroxisomes and supernatant from liver were prepared at various times after injection and the peroxisomes were treated to isolate

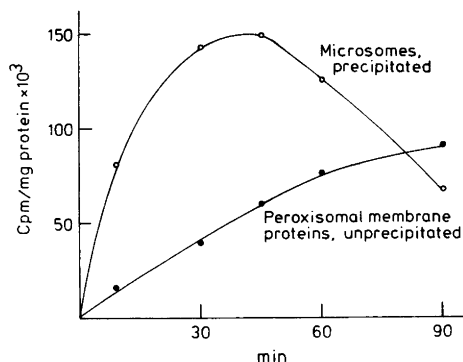


Fig. 1. Labeling of peroxisomal precursor proteins in microsomes and of peroxisomal membrane proteins. Rats were injected into the portal vein with 0.5 mCi [<sup>3</sup>H]amino acid mixture (Amersham) and after various times the liver was removed followed by preparation of microsomes and peroxisomes. The microsomes were precipitated by antibodies against peroxisomal membranes and adsorbed on a protein A-Sepharose column. The eluate and the peroxisomal membranes freed from the content were used to determine protein content and radioactivity.

membrane proteins free from the luminal content. There was no precipitation of any of the proteins in the supernatant. On the other hand, a part of the microsomal proteins reacted with antibodies and the radioactivity could be measured in the precipitate (Fig. 1). Highest specific activity was obtained after 45 min and after this time a decay occurred. Incorporation into total peroxisomal membrane proteins was considerably lower in the initial period and increased continuously in the first 90 min.

For closer identification of the proteins in the microsomal fraction which serve as precursors for peroxisomal membranes, *in vivo* labeling with injection of [<sup>35</sup>S]methionine and [<sup>35</sup>S]cysteine was performed. The microsomal membranes were precipitated with the antibody against peroxisomal membranes and after adsorption to protein A-Sepharose separation on slab gel was performed (Fig. 2). After 45 min 8 bands were visible on the autoradiographs ranging in MW between 30 000 and 90 000. Five of these bands possess high labeling while the remaining three gave low intensity band on the autoradiograph. After 90 min the radioactivity decreased considerably in all bands.

To follow the transport of phospholipids, rats were labeled *in vivo* with [<sup>3</sup>H]glycerol and

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Fig. 2. Slab gel of peroxisomal precursor proteins isolated from microsomes. Rats were injected in the portal vein with 0.5 mCi [ $^{35}\text{S}$ ]methionine and 0.5 mCi [ $^{35}\text{S}$ ]cysteine and the liver was removed after 5, 45, 90, or 180 min (lanes 1, 2, 3 and 4, respectively). Microsomes were precipitated with antibodies against peroxisomal membrane protein. The figure shows the result after autoradiography.

incorporation of this label into total phospholipid was followed (Fig. 3). Peak incorporation into microsomes occurred at 30 min followed by a rapid decay. As expected, the specific radioactivity in total peroxisomal phospholipid was lower at this time but increased continuously during the first two hour period. Since peroxisomes are considered to lack an enzyme system for synthesis of phospholipids, the experiments indicate relatively rapid transfer of lipids from endoplasmic membranes to peroxisomes.

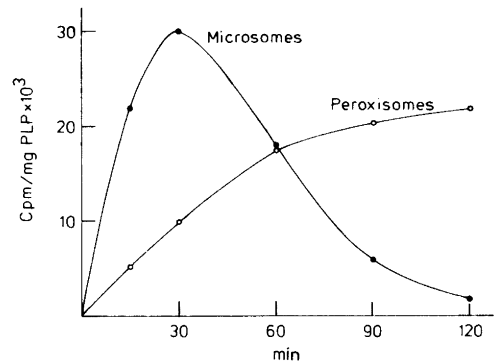


Fig. 3. Labeling of microsomal and peroxisomal phospholipids. Rats were injected in the portal vein with 0.5 mCi [ $^3\text{H}$ ]glycerol, the livers were removed after various times, microsomes and peroxisomes were prepared. The lipids were extracted with chloroform: methanol (2:1) and phospholipids were separated by chromatography on silicic acid column. The eluate was used to measure radioactivity and phospholipid.

Peroxisomal membranes were subjected to gel electrophoresis and few bands exhibited staining with Schiff's reagent, indicating the presence of carbohydrate residues. There are two possibilities for explaining the presence of protein-bound carbohydrates in peroxisomes: they are either synthesized at their final location or they are transported from the endoplasmic reticulum. The data presented in Table 1 demonstrate that both dolichol and dolichol-associated glycosylation reactions are present in peroxisomes.

The dolichol concentration is higher in peroxisomes than in microsomes and both dolichol mono- and pyrophosphatase activities are present in this fraction. In spite of the low activity of

Table 1. Dolichol and dolichol-associated reactions in peroxisomes. Dolichol and the various enzyme activities were measured as described earlier.<sup>4,5</sup>

	Peroxisomes Amount or activity	% of microsomal amount or activity
Dolichol <sup>a</sup>	0.85	163
Dolichol monophosphatase <sup>b</sup>	32	59.3
Dolichol pyrophosphatase <sup>b</sup>	69	32.4
NADPH-cytochrome <i>c</i> reductase <sup>c</sup>	0.002	1.9
UDP- <i>N</i> -acetylglucosaminyl transferase <sup>d</sup>	0.023	12.8
UDP-galactosyltransferase <sup>d</sup>	0.043	11.9

<sup>a</sup>  $\mu\text{g}/\text{mg}$  protein. <sup>b</sup> pmol Pi/[min(mg protein)]. <sup>c</sup>  $\mu\text{mol}/[\text{min}(\text{mg protein})]$ . <sup>d</sup> pmol/[min(mg protein)].

NADPH-cytochrome *c* reductase activity, which indicates a low microsomal contamination, both UDP-*N*-acetylglucosaminyl and UDP-galactosyl transferase activities are found at a level which cannot be explained by contamination with microsomal vesicles.

The experiments described in this paper indicate that some of the proteins of the peroxisomal membranes are synthesized in the endoplasmic reticulum and transferred through the cytoplasm to the peroxisomes. This transfer probably does not involve membrane flow and may involve only some individual proteins of the peroxisomal membranes. The cytoplasmic pool in this case is very minute and its precipitation with antibodies is not possible with currently available methods. Glycoproteins appear to be constitutive components of these membranes and the oligosaccharide chain is either synthesized here or completed by the dolichol mediated glycosyltransferase system.

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