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Rapid Report

Two yeast peroxisomal proteins crossreact with an antiserum against human sterol carrier protein 2 (SCP-2)

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An antibody raised against human sterol carrier protein 2 (SCP-2) crossreacts with two yeast peroxisomal proteins. These proteins have apparent molecular weights of 35 and 58 kDa. Subfractionation of peroxisomes revealed that the 58 kDa species is a soluble matrix protein, whereas the 35 kDa protein is membrane bound. Treatment of isolated peroxisomal membranes with 0.25 M KCl released the 35 kDa crossreactive protein into the soluble supernatant. However, lipid transfer activity could be attributed neither to the 35 kDa nor to the 58 kDa protein.

Eukaryotic cells contain lipid transfer proteins, which were shown to catalyze the translocation of lipids between donor and acceptor membranes in vitro. Three large groups of such proteins have been described according to their substrate specificity, namely phosphatidylinositol transfer protein (PITP), phosphatidylcholine transfer protein (PCTP), and non-specific lipid transfer protein (ns-LTP) (for recent reviews, see Refs. 1 and 2). The latter protein was shown to be identical to the so-called sterol carrier protein 2 (SCP-2), which in addition to all phospholipids is able to translocate sterols in vitro [3,4].

In the yeast, *Saccharomyces cerevisiae*, two lipid transfer proteins have been detected so far. One of these proteins is specific for phosphatidylinositol and phosphatidylcholine [5,6] and closely resembles the PITP from higher eukaryotes. The second yeast lipid transfer protein, named phosphatidylserine transfer protein (PSTP), is less specific and catalyzes the transfer of phosphatidylserine, phosphatidylethanolamine, cardiolipin, phosphatidic acid and ergosterol [7]. In contrast to the mammalian ns-LTP the yeast PSTP does not translocate phosphatidylinositol and phosphatidylcholine. Immunological crossreactivity between the PSTP isolated from yeast cytosol and the bovine ns-LTP could not be detected (Snoek, G., personal communication).

On the basis of cDNA cloning it was shown that the mammalian SCP-2 is a member of a family of proteins which share a common carboxy terminus [8–10]. A lower molecular weight species (13 kDa) was found in the cytosol, whereas a higher molecular weight form (58 kDa) is associated with peroxisomes [11].

Tan et al. [12] detected a protein named PXP-18 (molecular weight 13.8 kDa) in the peroxisomal matrix of the yeast *Candida tropicalis*, which exhibited lipid transfer activity. This protein showed 33% homology to the rat liver ns-LTP. Its participation in the β-oxidation of fatty acids was suggested. In the yeast, *Saccharomyces cerevisiae*, proteins homologous to mammalian ns-LTP/SCP-2 have not been detected so far. In this paper we present evidence that two proteins associated with peroxisomes of baker's yeast crossreact with an antiserum against human SCP-2. Subfractionation of peroxisomes revealed that one of the two crossreactive proteins is associated with the membrane, and the other is located in the matrix space.

The wild-type yeast strain, *Saccharomyces cerevisiae* D 273-10 B was grown under aerobic conditions in the presence of 0.1% oleic acid and 0.2% Tween 80 as described before [13]. A 30000 × *g* organelle pellet was prepared from spheroplasts [14] by differential centrif-

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ugation in a pH 6 breaking buffer [13,15] and fractionated either on a sucrose step gradient (30–60%, w/w) or on a Nycodenz step gradient consisting of equal volumes of 17, 24 and 35% Nycodenz in 5 mM Mes, 1 mM KCl, 0.24 M sucrose (pH 6) [15]. After centrifugation for 90 min in an SW-28 rotor (Beckman) at 26000 rpm peroxisomes were collected, diluted with breaking buffer and isolated by centrifugation at 34500 × *g* for 30 min.

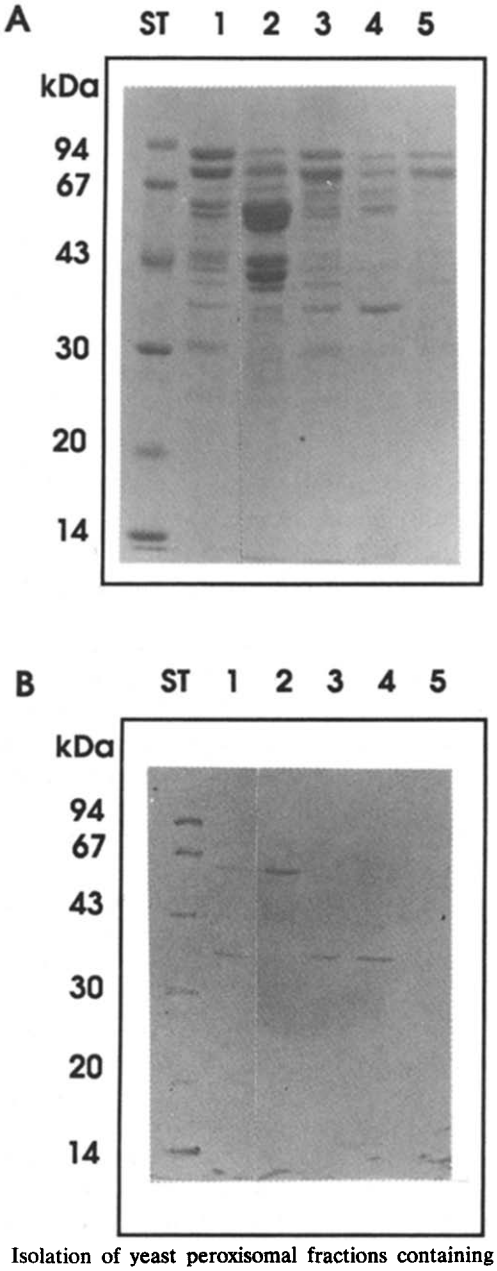

Fig. 1. Isolation of yeast peroxisomal fractions containing proteins crossreacting with an antibody against human SCP-2. (A) Proteins stained with Coomassie blue. (B) Western blot using antiserum against human SCP-2. ST, molecular weight standards; 1, peroxisomes; 2, peroxisomal matrix; 3, peroxisomal membranes; 4, 0.25 M KCl soluble peroxisomal membrane proteins; 5, 0.25 M KCl insoluble peroxisomal membrane proteins.

Isolated peroxisomes were hypotonically shocked for 1 h on ice in 10 mM Tris-HCl (pH 7.4) and frozen overnight. After thawing membranes and matrix were separated by centrifugation at 30000 rpm in a T-865 rotor (Sorvall) for 60 min. The resulting membrane pellet was washed once with 10 mM Tris-HCl (pH 7.4) and incubated for 10 min with 0.25 M KCl, 0.6 M mannitol. Insoluble material was collected by centrifugation at 30000 rpm for 60 min.

Yeast subcellular fractions were isolated and characterized as described by Zinser et al. [13]. Immunotitration using antisera against human SCP-2 (a gift from J. Billheimer, Wilmington, USA) and 3-oxoacyl-CoA thiolase (a gift from W. Kunau, Bochum, Germany) were performed following published procedures [16]. SDS-PAGE [17] and protein estimation [18] were carried out as described before. Lipid transfer assays using fluorescently labeled phospholipids [6] or radiolabeled substrates [5] were carried out as described elsewhere.

An antibody raised against human sterol carrier protein 2 (SCP-2) crossreacts with proteins of the yeast, *Saccharomyces cerevisiae*. When cells were grown under conditions appropriate to induce the proliferation of peroxisomes, the amount of two crossreactive proteins with apparent molecular masses of 35 and 58 kDa was increased several fold over the uninduced control. Both proteins were detected in the organelle pellet, but not in the cytosol. Upon subcellular fractionation the two crossreactive proteins were coisolated with peroxisomes (Table I; Figs. 1 A and B). Essentially no crossreactivity was observed with the plasma membrane, microsomes, mitochondria, vacuoles (see Table I) and a membrane fraction exhibiting similar sedimentation properties as induced peroxisomes, but obtained from non-induced cells (data not shown). These results together with enrichment factors of the two crossreactive proteins in comparison to marker proteins of peroxisomes, catalase and 3-oxoacyl-CoA thiolase, confirmed the peroxisomal localization of the 35 and 58 kDa protein.

Subfractionation of peroxisomes (Fig. 1 and Table II) revealed that the 58 kDa is matrix located as is catalase and 3-oxoacyl-CoA thiolase. The 35 kDa species is membrane associated. Treatment of isolated peroxisomal membranes with 0.25 M KCl solubilized the 35 kDa protein (Figs. 1 A and B, lanes 4; Table II). Removal of KCl from membrane washes led to a precipitation of the protein.

The function of SCP-2 related proteins in mammalian peroxisomes is still obscure. There is no proof that these organelle bound proteins are involved in lipid binding, lipid transport or related processes. In order to address the question as to the function of the two peroxisomal proteins of the yeast, *Saccharomyces cerevisiae*, which crossreact with the antibody against

TABLE I

Characterization of yeast subcellular fractions

Marker	Relative enrichment (fold) [a]				
	peroxisomes	plasma membrane	microsomes	mitochondria	vacuoles
Catalase [b]	4.7	n.d.	n.d.	0.2	–
Plasma membrane ATPase [c]	n.d.	80	1	0.2	> 0.1
Succinate dehydrogenase	0.5	0.2	0.02	4.2	> 0.02
NADPH: cytochrome-*c* reductase	0.3	0.9	6.8	0.4	1.3
α-D-Mannosidase	–	n.d.	0.8	–	27.3
35 kDa protein [c]	8	0.1	n.d.	0.05	n.d.
58 kDa protein [c]	10	n.d.	n.d.	n.d.	n.d.

[a] The specific activities of marker enzymes or the amount of marker proteins of the homogenate are set at 1. n.d., not detectable; –, not determined.

[b] Approximately one third of catalase activity in total homogenate is derived from the cytosolic isoenzyme.

[c] Determined by immunotitration.

human SCP-2, we measured lipid transfer activity in subfractions of peroxisomes. After partial purification neither the 35 kDa nor the 58 kDa crossreacting protein exhibited lipid transfer activity. The fact that aerobic growth of cells on oleic acid leading to the stimulation of peroxisomal proliferation, but not anaerobic growth in the presence of oleic acid and ergosterol stimulates the expression of these proteins (data not shown) also indicates that both crossreactive proteins are not simply lipid carriers in vivo. So far we are not able to assign any physiological role for these proteins, but investigations of their molecular properties will lead to a better understanding of their function in vivo.

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TABLE II

Enrichment of proteins crossreacting with an antibody against human SCP-2 in yeast peroxisomal fractions

	Relative enrichment (fold) [a]			
	catalase	3-oxoacyl-CoA thiolase [b]	35 kDa protein [b]	58 kDa protein [b]
Homogenate	1 [c]	1	1	1
Peroxisomes	4.7	10	8	10
Peroxisomal matrix	8.8	15	traces	70
Peroxisomal membranes	1.2	2	16	traces
0.25 M KCl soluble	2.0	n.d.	32	traces
0.25 M KCl insoluble	0.8	n.d.	traces	n.d.

[a] Data were obtained from three independent experiments. Specific activities of the homogenate were set at 1. n.d.: not detectable.

[b] Determined by immunotitration.

[c] Approximately one third of the catalase activity in total homogenate is derived from the cytosolic isoenzyme.

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