LOCALIZATION OF ACETYL-COENZYME A SYNTHETASE ON PEROXISOMES IN TETRAHYMENA

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The intracellular localization of acetyl-CoA synthetase was studied in the protozoan, Tetrahymena pyriformis. In cells grown under conditions which favor a conversion of acetate to carbohydrate via the glyoxylate bypass, this enzyme was found almost exclusively in the large particle fraction. The sedimentation pattern of this enzyme followed that of isocitrate lyase and lactate oxidase, but not of succinate dehydrogenase when the large particle fraction was subjected to further differential or density-gradient centrifugation. These results suggest that acetyl-CoA synthetase is localized on the peroxisome in Tetrahymena.

Isocitrate lyase and malate synthase, the key enzymes of the glyoxylate cycle, are localized on the peroxisomes (microbodies, glyoxysomes) in castor bean endosperm (1, 2) and in the protozoan, Tetrahymena pyriformis (3). Citrate synthase and isocitrate dehydrogenase are also present in this organelle in these organisms (2, 3), although not exclusively so. The presence of aconitase and malate dehydrogenase on the peroxisome has also been reported (2, 4).

In Tetrahymena, acetate can be rapidly and quantitatively converted to glycogen in cells grown under appropriate conditions (5-7). Since the initial steps of this conversion must presumably take place on the peroxisome, the intracellular localization of acetyl-Co A synthetase (acetate: Co A ligase, EC 6.2.1.1) in this organism was studied. The results suggest that this enzyme is localized primarily, if not exclusively, on the peroxisome, at least in cells grown under conditions that favor an active glyoxylate bypass.

Methods

Cultures of Tetrahymena pyriformis (E) were grown for 2 days under conditions which favor an active glyoxylate bypass (10). Washed cells were disrupted with a teflon pestle in a smooth walled tube in 0.25 M sucrose. A large particle fraction consisting of a tightly packed layer (Pt) and a fluffy layer (Pf) was collected by centrifuging the homogenate for 5 min. at 8000 x g. This was resuspended by homogenization and recentrifuged at the same speed. One ml of the Pt fraction was then layered over a gradient consisting of 3.0M (2 ml), 2.0M (4 ml), and 0.9M (2 ml) sucrose in a 100 x 15 mm plastic tube. The Pf fraction was not layered because it generally caused clumping. The tube was centrifuged for either 10 or 15 min at 6000 x g in the HB-4 swinging bucket rotor in a Sorvall RC-2 centrifuge. This procedure separates the particles primarily on the basis of differences in size. [The two particles can be separated by isopycnic centrifugation for 90 min at 100,000 x g (3). However, a centrifuge that could develop these forces was not available for this study.] Three bands were usually obtained and these were removed with a micropipette.

Acetyl-Co A synthetase (ACS) was assayed by the method of Berg (8), as described previously (9), except that dithiothreitol was substituted for glutathione. Procedures for the assay of isocitrate lyase (IL), lactate oxidase (LAO), and succinate dehydrogenase (SDH) have also been given (9-11).

Results

The distribution of ACS between the soluble (8000 x g, 10 min) and the particulate fractions of the cell is shown in Table I. Essentially all of the enzyme was recovered in the large particle fraction, as was SDH, which is localized exclusively on the mitochondrion (3, 12). Because the centrifuge available was incapable of developing forces sufficient

Table I. The percent of the total recovered enzyme found in the Pellet (8000 x g, 5 min), the Supernatant and the Wash of the pellet fractions.

	Acetyl-CoA Synthetase	Succinate Dehydrogenase	Protein
Pellet	93%	96%	51%
Supernatant	4%	3%	31%
Wash	2%	1%	17%
Recovery	106%	66%	99%

to separate mitochondria and peroxisomes on the basis of differences in density, we used several centrifugation schemes to achieve partial separation of the particles. Routinely, part of the washed P_t fraction was layered over a simple sucrose gradient, which was then centrifuged briefly at low speed (Table II). The highest specific activities of LAO and IL, which were used as marker enzymes for peroxisomes, were usually found in the top band. In contrast, that of SDH was quite low in this fraction. The activities of the two peroxisomal enzymes, but not of SDH were lowest in the bottom fraction. The distribution of ACS followed that of the peroxisomal enzymes. The distribution of these enzymes between the P_t and the P_f fractions is also shown in this table. The specific activities of LAO, IL, and ACS were highest in P_f, while that of SDH was highest in P_t.

In another experiment (Table II), the pellet fraction was subjected to hypotonic shock before being layered over the gradient. In this case, the relative specific activities of the peroxisomal enzymes and of ACS were highest in the middle band, but were approximately equal in all three fractions. In contrast, that of SDH was quite low in the top fraction and was highest in the bottom fraction.

Finally, a sample of P_t was layered over a more complex sucrose gradient, which was then centrifuged for 90 min at 11,100 x g (Table III).

Table II. Relative specific activity of enzymes in various subcellular fractions. The activity is expressed as the ratio of the specific activity of a given fraction to the average specific activity of all of the fractions. In experiment B, the particulate fraction was kept in distilled water for 30 min before being layered over the gradient.

Enzyme	Fraction							
			Experiment A			Experiment B		
	P_t	P_f	1	2	3	1	2	3
ACS	0.88	1.12	1.24	1.0	0.76	0.91	1.24	0.85
LAO	0.92	1.08	1.46	1.0	0.55	0.88	1.21	0.91
IL	0.90	1.10	1.66	0.87	0.45	0.96	1.22	0.83
SDH	1.27	0.73	0.19	1.63	1.18	0.32	1.25	1.43

Table III. Relative specific activities are expressed as in Table II. One ml of washed P_t was layered over a discontinuous sucrose gradient containing 2 ml each of 1.6, 2.2, 2.4, 2.6, 2.8, and 3.0M sucrose. Fraction 1 represents the topmost band, fraction 4 the lowest.

Enzyme	Fraction			
	1	2	3	4
ACS	0.19	1.64	1.21	0.96
LAO	0.11	1.47	1.21	1.24
SDH	0.26	0.81	1.20	1.73

While the peroxisomes apparently did not reach their equilibrium density (according to ref. 3 they should have banded below the mitochondria), there was some difference in the distribution of mitochondria and peroxisomes. Thus, the specific activity of SDH was highest in fraction 4, while that of LAO and of ACS were highest in fraction 2. SDH activity was relatively low in this fraction.

Discussion

Many enzymes of the Krebs and the glyoxylate cycles have now been found on the peroxisome in various plant and animal cells (1-4). In addition, glyoxysomes (a particle equivalent to the peroxisome) of castor bean endosperm contains the enzymes to carry out complete β-oxidation of long-chain fatty acyl-CoA esters (13). Thus, acetyl-CoA produced by this means is readily available for the enzymes of the glyoxylate cycle, which are also found on this organelle. Recently, we have studied some of the factors that control the levels of some of the peroxisomal enzymes (11).

ACS has been found in both the soluble (14) and the mitochondrial (15, 16) fractions of mammalian tissues. In yeast, the localization may vary, as the enzyme was found primarily in either the mitochondrial or the microsomal fraction, depending upon growth conditions and culture age (17).

It seemed likely that at least some of the ACS activity of Tetrahymena would be on the peroxisome. Citrate synthase and malate synthase, both of which require acetyl-CoA as one substrate, are found on this organelle, the latter exclusively so (3). In addition, cells grown under certain culture conditions can quantitatively convert acetate to glycogen (5, 7), implying that all of this substrate is metabolized at the peroxisome and that little or none reaches the oxidative enzymes of the mitochondria. The localization of ACS on the peroxisomes would be an effective means of channeling acetate into the glyoxylate bypass.

These results show that, under several test conditions, ACS sediments with two known peroxisomal enzymes, and that its sedimentation properties differ from those of SDH, which is known to be localized on the mitochondrion. Because only incomplete separation of particles was obtained by the procedures used here, it is not possible to determine if ACS is localized exclusively on the peroxisome. However, it seems

very unlikely that more than a small fraction of this enzyme could be localized on the mitochondrion. Since the cells used in these experiments convert acetate to glycogen at high rates, it is possible that the localization of ACS could be different in cells that can oxidize acetate. We are presently studying the localization in such cells.

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