PARTICULATE ENZYMES OF THE GLYOXYLATE CYCLE IN NEUROSPORA CRASSA

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SUMMARY : A particle carrying at least some of the enzymes of the glyoxylate cycle was found in Neurospora crassa. This particle has a density different from that of the mitochondrion. The proportion of the isocitrate lyase bound to this particle varies with the composition of the carbon source. The data suggest that this glyoxysome-like organelle carries part, if not all, of the constitutive isocitrate lyase. The "acetate-induced" lyase seems to appear in a soluble form which subsequently might become attached to the particle.

Neurospora crassa is able to utilize acetate as the only carbon source. The concomitant increase in isocitrate lyase suggests the funnelling of the 2-carbon molecule through the glyoxylate shunt (Turian 1960). The metabolic shift from the TCA to the glyoxylate cycle is still poorly understood. Among various possibilities, the specific association of the TCA and glyoxylate cycles to different organelles may provide a basis for a regulatory mechanism controlling the interplay of both cycles (Breidenbach and Beevers 1968).

Material and Methods

Neurospora crassa (wild type, strain Lindegren +), was grown for 4 1/2 days at 25 C in 7-1. batches of Westergaard-Mitchell liquid medium under vigorous aeration (Westergaard and Mitchell 1947). Unless otherwise stated, the carbon source was 1.5% acetate and 0.5% sucrose. After rinsing, the mat was dilacerated with razor blades, gently ground in a mortar and filtered through cheese cloth. The final dilution of the crude homogenate was 1 to 4 (w/v) in a medium containing : 0.4 M sucrose, 0.17 M TRICINE (pH 7.8), 10 mM KCl, 5 mM $MgCl_2$, 4 mM dithiothreitol. The fractionation included a low speed centrifugation (500 x g, 10 min.) followed by two centrifugations (8,000 x g, 40 min. and 48,000 x g, 40 min.) of the 500 x g supernatant. The pellets were layered on continuous sucrose gradients with 10 mM TRICINE (pH 7.8) and 1 mM cysteine. After ultracentrifugation (140,000 x g, 4 h.), the gradients were collected in 1 ml. fractions. TRITON-X was added to each fraction at a final concentration of 0.1%.

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Isocitrate lyase : 0.10 M phosphate buffer (pH 7.0), 5 mM $MgCl_2$, 2 mM EDTA, 2.4 mM phenylhydrazine, 14 mM D-L isocitrate (allo-free). Malate synthetase : incubation for 2-30 min. in 0.12 M TRIS buffer (pH 8.1), 5 mM $MgCl_2$, 1.82 mM acetyl-CoA, 15.2 mM neutralized glyoxylate; measure at 412 nm after addition of 0.12 mM dithiobis. Malate dehydrogenase : 0.10 M phosphate buffer (pH 7.6), 10 mM KCN, 0.12 mM NADH, 0.05 mM OAA. Succinate dehydrogenase : Hiatt 1961. NAD- and NADP dehydrogenases : Kobr et al. 1965 in presence of 10 mM KCN.

Results

The balance sheet of the activities recovered in the fractions obtained after low speed (500 x g) and high speed (48,000 x g) centrifugations is represented in Table I. In all cases the activities recovered in the pellets and supernatants are equal, within experimental errors, to those in the original crude homogenates. All enzymes measured in this experiment appear to sediment to some extent in the 48,000 x g pellets but not in the 500 x g pellet. The NAD isocitrate and malate dehydrogenases are found in both soluble and particulate forms. A limited but significant fraction of the NADP isocitrate dehydrogenase sediments in the 48,000 x g pellet and was found to be associated with the mitochondria. This result duplicates the observation of Müller et al. (1968) on Tetrahymena pyriformis. The isocitrate lyase and malate synthetase, both enzymes specific for the glyoxylate cycle, sediment somewhat in the 48,000 x g pellet. To test the

TABLE I

Balance sheet of activities
(activity in μmoles H^{-1})

	Crude homogenate	500 x g pellet	500 x g super	48,000 x g pellet	48,000 x g super
Isocitrate lyase	1,120	18	1,320	320	1,040
% of total	100	1.5	111	27	88
Malate synthetase	3,120	33	3,260	1,580	1,650
% of total	100	1.1	104	49	53
NAD ICDH	1,810	67	1,385	940	762
% of total	100	3.7	77	52	42
NADP ICDH	15,400	95	15,100	1,093	14,200
% of total	100	0.6	98	7	93
Malate dehydrogenase	256,000	2,940	291,000	45,540	210,000
% of total	100	1.1	113	18	82

TABLE II

Effect of washing on protein and isocitrate lyase contents of a 48,000 x g pellet from acetate-grown Neurospora

	Activity $\mu moles \cdot H^{-1}$		Proteins mg		Specific activity $\mu moles\ H^{-1} \cdot mg^{-1}\ prot.$	
	Expt. I	Expt. II	Expt. I.	Expt. II	Expt. I	Expt. II
Before washing	143	117	29.0	21.2	4.94	5.51
After washing	68	71	17.6	16.4	3.97	4.30

possibility of an accidental adsorption of isocitrate lyase in the pellet, its specific activities were compared before and after washing in 20 volumes of grinding medium. It appears (Table II) that approximately 50% of it can be washed out of the pellet. The washing also alters the stability of the particles since a substantial amount of proteins (20-40%) is solubilized from the pellet upon washing. The net result is a 20% decrease in the specific activity of the pellet, part of it resulting from enzyme denaturation since only 85% of the original activity was accounted for. This experiment shows that the binding of the isocitrate lyase to the particles is not an artifact of preparation. It also indicates that the particles are fairly labile. Therefore the percentages of activity recovered in the pellets represent minimal values.

Interestingly enough, the fraction of isocitrate lyase found in the pellet depends greatly on the carbon source provided in the nutrient medium. It amounts to 43% of the total when sucrose is provided alone but drops to 5-7% when acetate is the only carbon source (Table III). The intermediate value of 27% reported in Table II relates to a culture grown on

TABLE III

Effect of the composition of the carbon source on the distribution of isocitrate lyase (activity in $\mu moles \cdot H^{-1}$)

		Crude homogenate	500xg pellet	500xg super	48,000xg pellet	48,000xg super
2% sucrose	Activity	139	2.2	139	60	75
	% of total	100	1.4	100	43	54
2 % acetate	Activity	3,380	17.4	3,320	172	3,290
	% of total	100	0.5	100	5.1	98

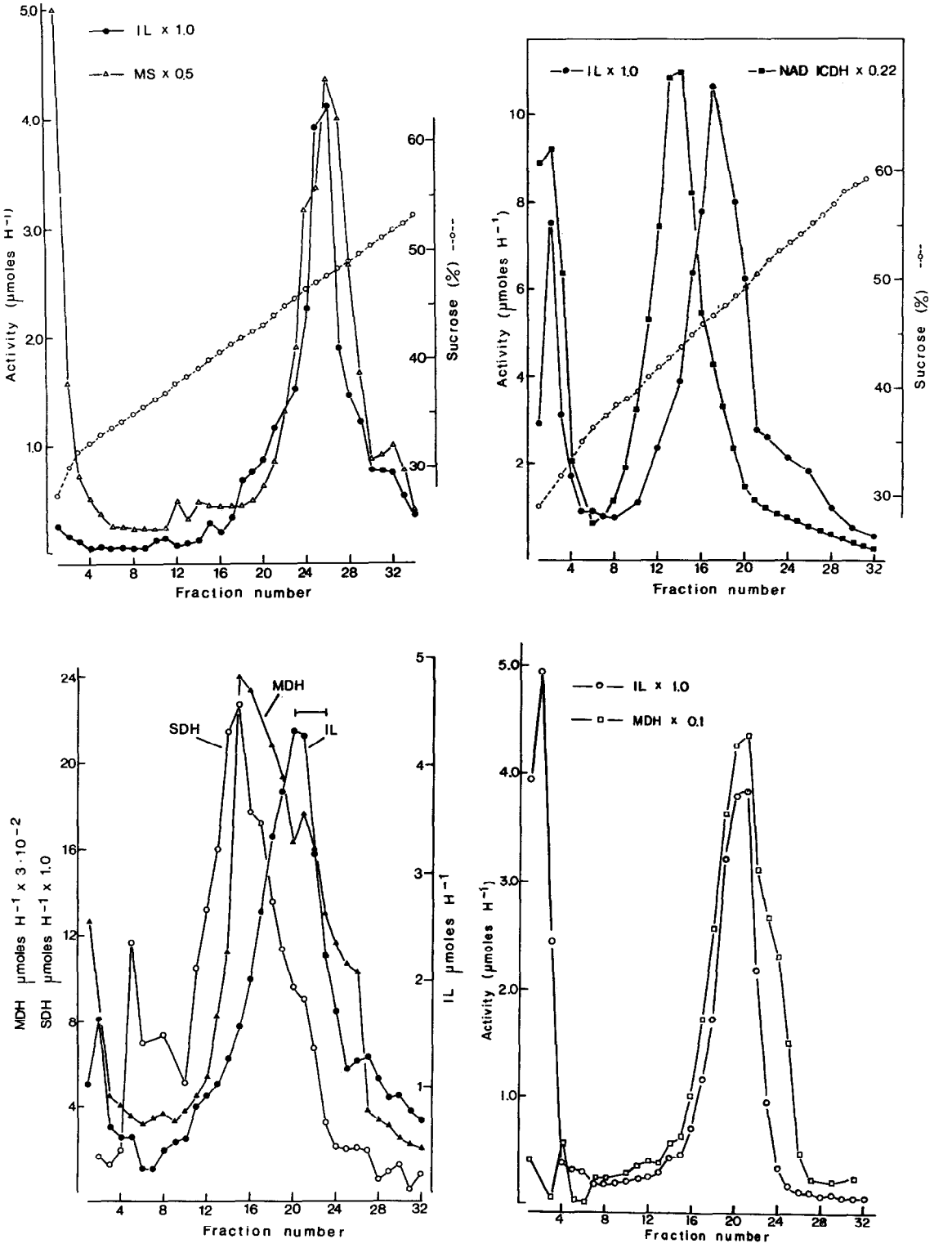

Fig. 1 Density distribution of enzymes associated with two particulate fractions from Neurospora crassa. IL : isocitrate lyase. MS : malate synthetase. NAD ICDH : NAD isocitrate dehydrogenase. SDH : succinate dehydrogenase. MDH : malate dehydrogenase.

a mixture of sucrose and acetate.

The distribution patterns of isocitrate lyase and malate synthetase in the sucrose gradient are identical, indicating their association to a common organelle (Figure 1, upper left). Both enzymes are distributed around a median density of 1.22 $g \cdot cm^{-3}$. In contrast, NAD isocitrate dehydrogenase peaks at a density of 1.19 $g \cdot cm^{-3}$ (Fig. 1 upper right). Even though the distributions of the dehydrogenase and of the lyase overlap extensively, the peaks of activities are located 4 tubes apart in the typical gradient shown here. Additional evidence for a particle distinct from the mitochondrion appears from the distributions of the succinate dehydrogenase and isocitrate lyase. (Figure 1, lower left). This gradient was made from particles which sedimented at 8,000 x g and contains a lighter species of mitochondria equilibrating at 1.16 $g \cdot cm^{-3}$. The malate dehydrogenase overlaps both succinate dehydrogenase and isocitrate lyase profiles. In order to decide whether or not the malate dehydrogenase is also associated with the particle carrying the isocitrate lyase, the fractions 20-24 were pooled and resedimented on another sucrose gradient. The similarity of the isocitrate lyase and malate dehydrogenase distribution (Figure 1, lower right) leads to the conclusion that the malate dehydrogenase is carried on the same particle which also contains the isocitrate lyase and the malate synthetase.

Discussion

The results show that at least some of the enzymes involved in the operation of the glyoxylate cycle in Neurospora are associated with a cellular organelle. But the proportion of isocitrate lyase which is particulate depends largely on the carbon source provided. Since growth on acetate in place of sucrose produces a 20-fold increase in the amount present in the tissue, it is concluded that the "acetate-induced" isocitrate lyase is not synthetized in these particles. The specific activity of isocitrate lyase found in particles extracted from acetate-grown Neurospora is 7 to 10-fold higher than that found in the mold grown on sucrose. This may indicate a progressive binding of the soluble enzyme to the particles. It is not known whether the soluble and particulate fractions exhibit different kinetics.

Despite extensive overlapping, the density distribution of enzymes markers of the TCA and glyoxylate cycles are significantly different. This eliminates the mitochondria as possible carriers of the glyoxylate cycle and indicates that the particle isolated from Neurospora crassa exhibits at least some of the characteristics of the glyoxysome found in the developing castor bean endosperm (Breidenbach and Beevers 1967).

The actual role of the particles isolated from Neurospora and their relationship with the mitochondria are under further investigation.

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References

Breidenbach, R. W. and H. Beevers, Biochem. Biophys. Res. Comm. 27 : 462 (1967)

Breidenbach, R. W. and H. Beevers, Plant Physiol. 43 : 705 (1968)

Hiatt, A. J., Plant Physiol. 36 : 552 (1961)

Kobr, M. J., G. Turian and Emma J. Zimmerman, Arch. Mikrobiol. 52 : 169 (1965)

Muller, M., J. F. Hogg and Ch. de Duve, J. biol. Chem. 243 : 5385 (1968)

Turian, G., Bull. Soc. Bot. Suisse 70 : 451 (1960)

Westergaard, M. and H. K. Mitchell, Amer. J. Bot. 34 : 573 (1947)