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Abstract

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Abstract: Prolonged sonication of prepared Neurospora crassa mitochondrial fractions disrupts and fractures the mitochondria inactivating the enzymes aconitase, succinic dehydrogenase and a diphosphopyridine nucleotide specific glutamic acid dehydrogenase (DPN-GAD). In contrast to this inactivation, a triphosphopyridine nucleotide specific glutamic acid drhydrogenase (TPN-GAD) is not affected.

Supporting Data: Mitochondrial fractions were obtained using a sand grinding technique (Stine, Master's thesis, Dartmouth College, 1963). Tubes containing the sand ground slurry were centrifuged at 500 g for 5 minutes and increased to 2000 g for 10 minutes. The supernatant (S-1) was carefully decanted and the precipitate of sand and cellular debris discarded. The major portion of the S-1 was recentrifuged at 8000 g for 30 minutes. The supernatants S-1, S-2 (8000 g) and the corresponding precipitate (PPT-2) were used in the sonication tests. These fractions were sonicated for a total of 8 minutes using an M.S.E. Mullard Ultrasonic Disintegrater equipped with an 0.9 cm diameter stainless steel probe and a temperature controlled sonicator cup (Hughes, J. Biochem. and Microbiol. Tech. and Eng. III, 405, 1961). Samples were withdrawn at 30 second intervals and aconitase, succinic dehydrogenase, TPN-GAD and DPN-GAD activity determined. Assays were run on a Cary model 14 recording spectrophotometer at the following wave lengths: TPN-GAD and DPN-GAD, 340 mμ; aconitase, 240 mμ; and succinic dehydrogenase, 429 mμ. A unit of enzyme activity is defined as a change in O.D. of 0.02 per minute (Barratt and Strickland, Arch. Biochem. Biophys. 102, 66, 1963). Activity is expressed as units per ml.

The results given in Table I are averages of triplicate tests on the S-1 and PPT-2, and a single test on the S-2 (each test was made on separately ground batches of freshly grown mycelia). In each case a sample was divided into 12 ml aliquots. The control was assayed at 0 and 8 minutes while the corresponding sonicated fractions were assayed at 30 second intervals through 8 minutes.

All 3 enzymes were found to be stable up to 8 minutes in the unsonicated controls. In sonicated material aconitase showed a decline in activity at 1 minute and a complete loss of activity in 4 to 6 minutes, and approximately a 50% loss of activity after 3 minutes. Succinic dehydrogenase appears to be more sensitive to sonication than aconitase. DPN-GAD is also sensitive to sonication with a complete loss of activity after 4 to 5 minutes of treatment. TPN-GAD is stable to sonication.

The low succinic dehydrogenase activity in the S-2 unsonicated material indicates the lack of mitochondria in the fraction. TPN-GAD shows equal activity in all fractions and therefore is probably not mitochrondrial bound. Since DPN-GAD is very sensitive to sonication (inactivation compares favorably with the inactivation of aconitase and succinic dehydrogenase) these data indicate that the DPN-GAD is a mitochondrial bound component.-- Department of Biological Sciences, University of Delaware, Newark, Delaware.

TABLE I

Ultrasonication effects on aconitase, succinic dehydrogenase, DPN-GAD and TPN-GAD of Neurospora crassa mycelia.

	# of tests	Control		Sonication Time in Minutes Units per ml.							
Aconitase		0 to 10 min.		1	2	3	4	5	6	7	8
S-1	3	26	26	23	20	15	10	0	0	0	0
S-2	1	25	25	20	14	3.1	2	1	0	0	0
PPT-2	3	26	26	23	20	10	0	0	0	0	0
Succinic Dehydrogenase											
S-1	3	1.5	1.5	1	1	0.3	0	0	0	0	0
S-2	1	0.8	0.64	0	0	0	0	0	0	0	0
PPT-2	3	6.1	6.0	3	2	0.7	0.2	0	0	0	0
DPN-GAD											
S-1	3	50	50	40	20	5	0	0	0	0	0
S-2	1	60	60	46	38	17	3	0	0	0	0
PPT-2	3	95	95	80	50	20	0	0	0	0	0
TPN-GAD											
S-1	3	500	485	485	500	550	550	550	550	550	550
S-2	1	580	580	580	580	580	580	580	580	580	580
PPT-2	3	8.4	8.1	8.1	8.3	8.5	9.5	9.5	9.5	9.5	9.5