Effect of ultrasonication on selected enzymes from Neurospora crassa mycelia

G. J. Stine

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Abstract
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EDTA and with 20 x 10^-5 M 8-HQ over the untreated crosses. With respect to region I of linkage
group I, a significant increase in the cross-over frequency is shown in the crosses treated with
4 x 10^-5 M EDTA and with 30 x 10^-5 M 8-HQ. Whereas a significant increase in the cross-over fre-
quency is observed in region II of linkage group I among crosses treated with 4 x 10^-5 M, 10 x 10^-5 M
and 20 x 10^-5 M EDTA, a significant decrease is indicated in a cross treated with 10 x 10^-5 M 8-HQ.

Different concentrations of the chelating agents appears to have a variable pattern of modifications
in the cross-over values among chromosomal segments. This may be a reflection of their differential
effects on the general inter-cellular ionic environment bringing about an interaction at the macro-
molecular level, in such a way that it causes modifications in genetic exchanges. Further investigations,
however, may reveal whether by the treatment with the specific molar concentrations of chelating agents,
the hypothetical links of the macromolecular complexes of component nucleic acids and proteins are
weakened or a chain of physiological processes directed towards the ultimate modifications in the
activity of genetic material are initiated—Department of Botany, University of Malaya, Kuala Lumpur,
Malaya.

Stine, G. J. Effect of ultrasonication

on selected enzymes from Neurospora

crassa mycelia.

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 tri-
phosphopyridine nucleotide specific glutamic acid dehydrogenase (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 Disintegrator equipped with a 0.9 cm diameter stainless steel probe and a temperature
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 mu; aconitase, 240 mu; and succinic dehydrogenase,
429 mu. 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 correspond-
ing 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 mito-
chondria in the fraction. TPN-GAD shows equal activity in all fractions and therefore is probably not
mitochondrial 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.
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<th># of tests</th>
<th>Control</th>
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<td></td>
<td></td>
<td></td>
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<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>S-1</td>
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