AMINOACETONE SYNTHETASE OF LIVER MITOCHONDRIA

Thesis submitted for the degree

of

Master of Science

by

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from

The Department of Biochemistry.

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STATEMENT

This thesis contains no material previously submitted for any other degree or diploma in this or any other University. To the best of my knowledge and belief, this thesis does not contain any material previously published or written by any other person, except where due reference is made in the text.

Robert L. Walsh.

PUBLICATIONS

Walsh, R. L. and Elliott, W. H. (1969) Aminoacetone synthetase of liver mitochondria; its isolation and properties. Proc. Aust. Biochem. Soc., P.86.

Irving, E. A., Elliott, W. H., Whiting, M. J. and Walsh, R. L. (1970) δ -Aminolevulinic acid and amino-acetone synthetases from liver mitochondria. J. Biol. Chem. (accepted for publication 1970).

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SUMMARY

1. An assay procedure for the determination of aminoacetone synthetase activity is described. The method depends upon the generation of acetyl Coenzyme A from acetyl phosphate and Coenzyme A by *E. coli* phosphotransacetylase.

2. High levels of the enzyme have been found in the mitochondria of the livers of guinea pig, ox, sheep, pig, lamb and calf.

3. Aminoacetone synthetase was found to be located on the inner mitochondrial membrane.

4. Aminoacetone synthetase from sheep liver was solubilised.

5. The solubilised enzyme is stable in concentrated protein solution but very unstable in a diluted form. The enzyme can be stabilised by the addition of pyridoxal-5-phosphate, ethylene diamine tetra-acetic acid and inexplicably by serine or aspartic acid.

6. A partial purification procedure is described which achieves an almost eighty-fold purification of the enzyme from the mitochondria.

7. Partial purification of the E. coli phosphotransacetylase resulted in a loss of aminoacetone formation when it was included in the reaction mixture containing partially purified aminoacetone synthetase. However, partially purified phosphotransacetylase was active with crude preparations of aminoacetone synthetase. No explanation for these results has been found.

8. Aminoacetone synthetase is inhibited by coenzyme A. The non-linear time course of aminoacetone formation obtained, when using added acetyl coenzyme A as substrate is almost certainly due to the coenzyme A released in the assay inhibiting the enzyme.

9. A number of agents were examined in their effect on aminoacetone formation in an assay using added acetyl coenzyme A. Aminoacetone synthetase was rather surprisingly inhibited by heme.

ABBREVIATIONS

AA	aminoacetone
AA pyrrole	2, 4-dimethyl-3-acetyl pyrrole
AIA	allyl isopropyl acetamide
ALA	δ-aminolevulinic acid
ALA pyrrole	2-methyl-3-acetyl-4-propionic acid pyrrole
AMP	adenosine monophosphate
ATP	adenosine triphosphate
cm	centimeter
CM	carboxymethyl
CoA	coenzyme A
DEAE	diethyl amino ethyl
DDC	3,5-dicarbethoxy-1,4-dihydro-collidine
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetra-acetic acid, disodium salt
gm	gram
GDP	guanosine diphosphate
GTP	guanosine triphosphate
M	molar
mg	milligram
ml	millimeter
mM	millimolar

ug	microgram
umole	micromole
NAD	nicotine adenine dinucleotide
NADH	nicotine adenine dinucleotide, reduced
PO_4	phosphate
RNA	ribose nucleic acid
TCA	trichloracetic acid
tris	Tris (hydroxy methyl) aminomethane
UDP	uridine diphosphate
UTP	uridine triphosphate

INDEX