THE OXIDATION OF LINOLEATE AND OTHER LONG-CHAIN FATTY ACIDS IN RAT AND SHEEP LIVER MITOCHONDRIA

A thesis presented in partial fulfilment
of the requirement for the degree of
Doctor of Philosophy
in Biochemistry
at
Massey University
New Zealand

John Campbell William Reid
1986

LIBRARY CLEARANCE

1. (a) I give my permission for my thesis, entitled

THE OXIDATION OF LINOLEATE AND OTHER LONG-CHAIN FATTY ACIDS IN RAT AND SHEEP LIVER MITOCHONDRIA

to be made available to readers in the Library under the conditions determined by the Librarian.

(b) I agree to my thesis, if asked for by another institution, being sent away on temporary loan under conditions determined by the Librarian.

(c) I also agree that my thesis may be copied for Library use.

2. ~~I do not wish my thesis, entitled~~

~~.....~~

~~.....~~

~~.....~~

~~to be made available to readers or to be sent to other institutions without my written consent within the next two years.~~

Signed ..... J Reid

Date 1/10/84

~~Strike out the sentence or phrase which does not apply.~~

The Library
Massey University
Palmerston North, N.Z.

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

Name and Address Date

.....

.....

.....

.....

.....

Abstract

Sheep liver mitochondria oxidised palmitate, oleate and linoleate at slower rates than did rat liver mitochondria. Rat liver mitochondria oxidised linoleate at 1.2 to 1.7 times the rate observed with palmitate as the substrate. However, sheep liver mitochondria oxidised linoleate at 0.74 to 0.84 the rate observed when palmitate was the substrate. The biochemical basis of this difference is not understood.

The reaction catalysed by the enzyme carnitine acyltransferase I is believed to be an important regulatory step in the oxidation of long-chain fatty acids and is known to be competitively inhibited by malonyl-CoA. Both rat and sheep liver mitochondria were able to form acyl carnitine when palmitoyl-CoA and linoleate, coupled with an acyl-CoA generating system, were the acyl substrates.

Malonyl-CoA was very effective in inhibiting the CAT I reaction in sheep liver mitochondria. When linoleate, coupled with an acyl-CoA generating system, was the substrate for CAT I, 1 μM malonyl-CoA was found to inhibit the reaction by 90%. However, when the same substrate was assayed in rat liver mitochondria the inhibition was much less, 22 μM malonyl-CoA leading to only 50% inhibition of the CAT I enzyme. When palmitoyl-CoA was used as a substrate for the enzyme CAT I, little difference was seen between rat and sheep liver mitochondria in the extent of inhibition observed over the concentration range of 1 to 5 μM malonyl-CoA.

These experiments indicate that sheep liver mitochondria could oxidise palmitate rather than linoleate at low levels of malonyl-CoA, as one might expect *in vivo*. In contrast, in rat liver mitochondria, linoleate would be oxidised faster than palmitate at all concentrations of malonyl-CoA investigated.

It is suggested that this system may be an important means whereby sheep are able to conserve linoleate by preventing its oxidation.

In addition the mitochondrial glycerol 3-phosphate acyltransferase reaction was investigated with both sheep and rat liver mitochondria. With linoleate and an acyl-CoA generating system, rat liver preparations esterified 1.5 nmoles min/mg protein whereas sheep liver mitochondria esterified less than one tenth of this. It was concluded esterification of linoleate to glycerol 3-phosphate is not an important mechanism of conserving linoleate in sheep liver mitochondria.

Esterification of palmitate to glycerol 3-phosphate was studied using palmitoyl-CoA as the acyl donor. At maximal rates of esterification it was observed that rat liver mitochondria esterified palmitoyl-CoA at 2 nmoles/min/mg whereas sheep mitochondria esterified 0.8 nmoles/min/mg.

Acknowledgement

I wish to thank my supervisor Dr D.R. Husbands for his advice, encouragement and sense of humour throughout this work.

I would also like to thank Dr J.C. Hawke for his advice throughout this work especially in the writing of this thesis.

I am also grateful to Earl Stevens for his help with statistical analysis and for his general support.

An extended thanks is given to members of the Chem/Biochem department and to many other friends for their support throughout this work.

Abbreviations

CAT	-	carnitine acyltransferase
CPT	-	carnitine palmitoyltransferase
GPAT	-	glycerol 3-phosphate acyltransferase
FFA	-	free fatty acid
DTT	-	dithiothreitol
PPO	-	2,5-diphenyloxazole
POPOP	-	1,4-bis[2-(5-phenyloxazolyl)]benzene
NbS_2	-	5,5'-dithiobis-(2-nitrobenzoic acid)
EGTA	-	ethyleneglycol-bis-(β-amino-ethyl ether) N,N'-tetra-acetic acid
g	-	gravitational force
g	-	grams
l	-	litre
16:0	-	palmitic acid
18:1		oleic acid
18:2	-	linoleic acid

List of Contents

Page

Page

List of Tables

List of Figures