MECHANISTIC STUDIES OF ENZYMATIC REACTIONS INVOLVED IN STRAIGHT AND BRANCHED CHAIN FATTY ACIDS OXIDATION

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Abstract

Fatty acids (FAs) belong to a physiologically important class of molecules involved in energy storage, membrane structure, and various signaling pathways. The oxidation of fatty acids is a central metabolic process providing electrons to the respiratory chain and thus energy for a multitude of needs in aerobic organisms. It is a complex process involves more than a dozen enzymes and must be carefully regulated. The consequences of dysfunctions in these enzymes can be many-fold and severe with regard to human health, and thus an understanding of their basic mechanisms is of great relevance. L-3-Hydroxyacyl-CoA dehydrogenase (HAD) and mitochondrial trifunctional protein (MTP) are enzymes that take part in the β-oxidation in mitochondria. This is the major process of fatty acids oxidation, and these enzymes catalyze the oxidation of straight chain fatty acids. Short/branched chain acyl-CoA dehydrogenase (SBCAD) and isovaleryl-CoA dehydrogenase (IVD) catalyze the oxidation of branched chain fatty acids from the catabolism of amino acids. Their properties and catalytic mechanisms have been studied by several research groups.

L-3-Hydroxyacyl-CoA dehydrogenase (HAD) is the penultimate enzyme in the β-oxidation spiral, catalyzing the oxidation of the hydroxyl group of L-3-hydroxyacyl-CoA to a keto group, concomitant with the reduction of NAD^+ to NADH. Ser137 and Asp208 are highly conserved residues in HAD from various sources. In the present study, site-directed mutagenesis was carried out, which identified that they were important residues involved in the catalysis and supported the speculation based on its crystallographic structure. Ser137 and Asp208 should form hydrogen bond with the substrate, respectively, to stabilize the orientation of

substrate in the active site. A series of substrate analogs were designed to probe the catalytic mechanism of HAD. A new mechanism involving the formation of enolate intermediate was proposed. In addition, two salt bridges between Glu117 and Arg209, which are located in two monomers respectively, were proposed to stabilize the dimer based on the information from the three-dimensional structure. The mutagenesis study of Glu117 showed the disruption of the salt bridges do not affect the formation and stability of the dimer. The mutation of Arg209 affected the correct folding of HAD to yield inclusion bodies. This indicated that the conserved Arg209 was crucial in maintaining protein structure.

Short/branched chain acyl-CoA dehydrogenase (SBCAD) and isovaleryl-CoA dehydrogenase (IVD) are enzymes involved in oxidation of branched chain fatty acids, which are from catabolism of isoleucine and leucine. The comparative studies of two enzymes on substrate specificity with various substrate analogs were carried out. IVD displayed broader substrate spectrum including the branched chain and straight chain substrates. 2-Octynoyl-CoA and 2-octy-4-enoyl-CoA were found to be mechanism-based inhibitors of SBCAD, but not inhibitors of IVD. The inactivation mechanism was proposed to involve a nucleophilic attack through Michael addition. Moreover, SBCAD was found to have intrinsic enoyl-CoA isomerase activity, which was confirmed by HPLC analysis. Gly260 was confirmed to be critical for the isomerase activity of the enzyme too. This residue might maintain the correct orientation of substrate via a hydrogen bond, which is consistent with the conclusion from the study of enoyl-CoA isomerase. IVD did not show isomerase activity, but displayed intrinsic enoyl-CoA hydratase activity, which was confirmed by HPLC analysis. The mutagenesis studies supported Glu254 was the catalytic residue for both dehydrogenase and hydratase activities.

Mutations at Glu381 and/or Gly260 introduced the hydratase activity in SBCAD, which is absent in wild-type SBCAD.

Mitochondrial trifunctional protein (MTP) catalyzes the last three steps of the β-oxidation of long-chain fatty acids. This enzyme complex is composed of four α and four β subunits. The 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities reside on the α-subunit, whereas the 3-ketoacyl-CoA thiolase activity is located on the β-subunits. This enzyme complex is bound to the mitochondrial inner membrane. A lot of efforts were made to gain the active recombinant protein in order to study its property and mechanism. The α-subunit and β-subunit were expressed separately in bacterial expression system and yeast expression system. In the yeast expression system, the soluble α-subunit and β-subunit could be detected by Western blotting using anti-His-tag antibody. However, no enough amount of protein could be obtained. In the bacterial expression system, pLM1, pET-32a(+), and pMIS3.0E vector were tested. pMIS3.0E vector has a nucleotide sequence of Mistic, which was found to increase the solubility of expressed proteins. Soluble β-subunit fusion protein was obtained, and the V_{max} value of 1.7 μmol/mg/min and K_M value of 30.5 μM were determined with 3-ketooctanoyl-CoA. Unfortunately, the α-subunit was still not soluble using pMIS3.0E vector.

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List of Abbreviations

Å	ångström
AACoA	acetoacetyl-CoA
AcAcCoA	acetoacetyl-CoA
ABC	ATP-binding cassette
ACADSB	gene of short/branched chain acyl-CoA dehydrogenase
ACBP	acyl-CoA-binding protein
ACD	acyl-CoA dehydrogenase
ACAD	acyl-CoA dehydrogenase
ACS	acyl-CoA synthetase
AD	Alzheimer's disease
ADP	adenosine diphosphate
AMP	Adenosine monophosphate
AOX	alcohol oxidase
ATP	adenosine triphosphate
B-factor	temperature factor
bp	base pair(s)
BSA	bovine serum albumin
4-CBA	4-chlorobenzoyl coenzyme A
CD	circular dichroism
cDNA	complementary deoxyribonucleic acid
Cn	carnitine
CoA	coenzyme A
CPT	carnitine palmitoyltransferase
C-terminus	carboxyl terminus
DCIP	2,6-dichlorophenol indophenol
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECH	2-enoyl-CoA hydratase
ECI	Δ^3-Δ^2-enoyl-CoA isomerase
E. coli	*Escherichia coli*

EDTA	ethylenediaminetetraacetate
ER	endoplasmic reticulum
ETF	oxidized electron transfer flavoprotein
ETFH	reduced electron transfer flavoprotein
FA	fatty acids
FABP	fatty acid binding protein
FAD	flavin adenine dinucleotide (oxidized form)
FPLC	fast protein liquid chromatography
GBP	gastrin-binding protein
GCAD	glutaryl-CoA dehydrogenase
GC-MS	gas chromatography/mass spectrometry
GFP	green fluorescent protein
HADHA	the gene of the α-subunit of MTP
HADHB	the gene of the β-subunit of MTP
HADHSC	the gene of L-3-hydroxyacyl-CoA dehydrogenase
4-HBA	4-hydroxybenzoyl coenzyme A
HAD	L-3-hydroxyacyl-CoA dehydrogenase
HPLC	high-performance liquid chromatography
IBD	isobutyryl-CoA
IPTG	isopropyl β-D-1-thiogalactopyranoside
IVA	isovaleric acidemia
IVD	isovaleryl-CoA dehydrogenase
kb	kilobase(s)
KT / KAT	3-ketoacyl-CoA thiolase
kDa	kilodalton
LCAD	long-chain acyl-CoA dehydrogenase
LCHAD	long-chain 3-hydroxyacyl-CoA dehydrogenase
2MBCD	2-methylbutyryl-CoA dehydrogenase
MCAD	medium chain acyl-CoA dehydrogenase
MCPA-CoA	methylenecyclopropylacetyl-CoA
MFE-1, -2	multifunctional enzyme type 1, type 2
MIM	mitochondrial inner membrane
mmBCFAs	monomethyl branched-chain fatty acids

MS/MS	tandem mass spectrometry
MTP / TFP	mitochondrial trifunctional protein
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
NCS	noncrystallographic symmetry
NSAID	nonsteroidal anti-inflammatory drug
nt	nucleotide(s)
N-terminus	amino terminus
ORE	oleate response element
ORF	open reading frame
PCR	polymerase chain reaction
PD	Parkinson's disease
PDB	protein data bank
pFOX	partial fatty acid oxidation
PHHI	persistent hyperinsulinemic hypoglycemia of infancy
PMS	phenazine methosulfate
PPi	pyrophosphate
RC	respiratory chain
RNA	ribonucleic acid
SBCAD	short/branched chain acyl-CoA dehydrogenase
SBCADD	short/branched chain acyl-CoA dehydrogenase deficiency
SCAD	short chain acyl-CoA dehydrogenase
SCEH	short chain enoyl-CoA hydratase
SCHAD	short chain 3-hydroxyacyl-CoA dehydrogenase
SDR	short-chain alcohol dehydrogenase/reductase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPA-CoA	spiropentylacetyl-CoA
TEA	triethanol amine
UTR	untranslated region
UV	ultraviolet
VLCAD	very long-chain acyl-CoA dehydrogenase

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