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Mechanistic Studies of Enzymatic Reactions Involved in Fatty Acid Oxidation

參與脂肪酸代謝的酶的反應機理研究

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Abstract

Numerous diseases have been reported in relation to fatty acids, such as cardiovascular disease, cancer, diabetes, rheumatoid arthritis, fibrosarcoma-induced hyperlipidemia, etc. The regulation of fatty acid oxidation has been reported as a potential method treating non-insulin dependent diabetes mellitus (NIDDM) and inhibitors of enzymes involved in the metabolism of fatty acids have been synthesized and studied as potential medicines. Medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA oxidase (ACO) and 3-ketoacyl-CoA thiolase (KT) are three key enzymes involved in the β-oxidation of fatty acid. In the present study, we found that both MCAD and ACO have intrinsic isomerase activities, and carried out extensive studies of MCAD, ACO, and KT through site-directed mutagenesis and incubation with various substrate analogs followed with analysis.

We cloned the genes of rat acyl-CoA oxidase, 3-ketoacyl-CoA thiolase and medium-chain acyl-CoA dehydrogenase into a bacterial expression vector pLM1 with six continuous histidine codons attached to the C or N-terminal of the genes respectively. The three cloned genes were overexpressed in *Escherichia coli* and the soluble proteins were purified with a Hitrap chelating metal affinity column in over 90% yield to apparent homogeneity. MCAD and ACO were found to have intrinsic enoyl-CoA isomerase activity, which were confirmed using incubation followed with HPLC analysis. E376 mutants of MCAD were constructed, and it was shown that E376 is the catalytic residue

for both dehydrogenase and isomerase activities of the enzyme. E421 mutants of ACO were also constructed, and it was shown that E421 is the catalytic residue for both oxidase and isomerase activities of the enzyme. MCAD and ACO may function as isomerase in vivo when authentic isomerase is deficient. As we know, this is the first report that MCAD and ACO have intrinsic enoyl-CoA isomerase activity.

Four MCAD mutants Y375A, Y375E, Y375R and Y375K were constructed, and it was found that the mutant Y375K showed intrinsic acyl-CoA oxidase activity, which can transfer electrons to molecular oxygen. 2-Octy-4-enoyl-CoA was found to be a mechanism-based irreversible inhibitor of MCAD, and the mechanism of inactivation was different from those previous reported for known MCAD inhibitors. 2-Octe-4-ynoyl-CoA and 2-pente-4-ynoyl-CoA were both found to be mechanism-based inhibitors of ACO. ACO mutants Y232 and Y401 were constructed for studying the importance of the two residues, and it was found that Y232 and Y401 were important in cofactor FAD binding through kinetic and spectrum studies. Four KT mutants H352A, H352E, H352K and H352Y were constructed, and it was found that all mutants have significantly decreased activity, which confirmed H352 is an essential catalytic residue. KT mutant S251 was also constructed, and it was shown that residue S251 plays an important role in substrate binding. 2-Octynoyl-CoA and 2-octy-4-enoyl-CoA were both found to be mechanism-based inhibitors of KT.

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Abbreviations

Amino acids:

A	Ala, alanine
C	Cys, cysteine
D	Asp, aspartic acid
E	Glu, glutamic acid
G	Gly, glycine
H	His, histidine
K	Lys, lysine
R	Arg, arginine
Q	Gln, glutamine
T	Thr, threonine
Y	Tyr, tyrosine

Å	ångström
ABC	ATP-binding cassette
ACBP	acyl-CoA-binding protein
ACO	acyl-CoA oxidase
ADP	adenosine diphosphate
ATP	adenosine triphosphate
B-factor	temperature factor
CD	circular dichroism
cDNA	complementary deoxyribonucleic acid
CoA	coenzyme A
CPT	carnitine palmitoyltransferase
C-terminus	carboxyl terminus

DCPIP	2,6-dichlorophenolindophenol
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ECH	enoyl-CoA hydratase
ECI	enoyl-CoA isomerase
EDTA	ethylenediaminetetraacetate
ETF	electron transferring flavoprotein
FAD	flavin adenine dinucleotide (oxidized form)
GBP	gastrin-binding protein
GFP	green fluorescent protein
HPLC	high-performance liquid chromatography
IPTG	isopropyl-β-D-thiogalactopyranoside
KDa	kilodalton
KT	3-keto-acyl-CoA thiolase
MCAD	medium chain acyl-CoA dehydrogenase
MCPF-CoA	methylenecyclopropylformyl-CoA
MFE-1, -2	multifunctional enzyme type 1, type 2
MES	2-(N-morpholino)ethanesulfonic acid
MTP	mitochondrial trifunctional protein
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NCS	noncrystallographic symmetry
NIDDM	non-insulin dependent diabetes mellitus
N-terminus	amino terminus
PCR	polymerase chain reaction
PMS	phenazine methosulfate
PTS	peroxisomal targeting signal
RNA	ribonucleic acid

SCP	sterol carrier protein
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPA-CoA	spiropentylacetyl-CoA
TEA	triethanol amine
UV/Vis	ultraviolet-visible spectroscopy