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Studies of the Enzymes Involved in Unsaturated Fatty Acid Oxidation
參與不飽和脂肪酸氧化的酶的研究

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Abstract

Polyunsaturated fatty acids (PUFA) are important component of mammalian diets, and its beneficial effects on human development, cardiovascular health and diabetes have been well documented. To further understand the mechanism of the unsaturated fatty acids metabolism may provide useful information for learning the relationship between PUFA and diseases, and might give further information for the treatment. There are three auxiliary enzymes required for unsaturated fatty acids oxidation in mammals, including Δ^3-Δ^2-enoyl-CoA isomerase (ECI), $\Delta^{3,5}$-$\Delta^{2,4}$-dienoyl-CoA isomerase (DECI), and 2,4-dienoyl-CoA reductase (DECR). Since ECI and DECI belong to the same mechanistically diverse family, crotonase-like family, they were grouped together for the structure and function study, with another member of this family, 2-enoyl-CoA hydratase (ECH). Human mitochondrial DECR (mDECR) was studied separately.

A highly conserved salt bridge between the N-terminal core domain and the C-terminal domain exists in the members of the crotonase-like family. The mutagenesis study of this salt bridge was carried out in ECI, ECH and DECI. It is interesting that a single mutation of the salt bridge residue would introduce ECH activity in ECI which is absent in the wild type ECI and

enhance the residual ECH activity of DECI. It is the first report in crotonase-like family that a single substitution resulted in occurring of promiscuous activity. Further structure studies showed the assembly modes of the three enzymes are different, especially at the subunit-subunit interfaces, which is closely related with some active site residues. The salt bridge residues, although far away from the active site, plays an important role in maintaining the interface contacts of ECI, and indirectly related with the active site of the adjacent subunit. ECI mutant K242 (ECIm_K242) mutants showed much higher affinity for the isomerization product, 2-hexenoyl-CoA, than for its original substrate, 3-hexenoyl-CoA.Therefore, 2-enoyl-CoA could not be released from the active site very quickly. Once the catalytic residue deprotonates the water molecule that is positioned just right for nuleophilic attack at the Cα of the product, the hydration reaction might occur before its release into the solution. The study of DECI also supports that the interface contact plays an important role in maintaining the substrate binding pocket and/or active site conformation. Quaternary structure of protein has not been an hot field for the protein function study, however, reports showed that alterations in subunit interactions will influence protein structure or dynamics and, thereby, affect catalysis. Therefore, the inter-subunit interactions might also be a quick regulation position for the *in vivo* metabolic control, thereby might also be a potential target position for drug and other small molecules.

Another part of the present study was the mutagenesis study of human mitochondrial dienoyl-CoA reductase. Human mDECR belongs to the Short-

chain Dehydrogenase/Reductase (SDR) superfamily. Six active site residues, which might be directly involved in the catalysis based on comparison with the structure of other members of SDR superfamily, were chosen for mutagenesis study. The results showed DECR, though similar, is distinct from other enoyl-thiolester reductases. Five highly conserved acidic residues were also mutated to alanine to study their possible roles in human mDECR. The mutation of different position affected either the binding of the cofactor and the substrate or the catalytic efficiency of the enzyme. It might suggest that the interface contacts between subunit A and B play an important role for the catalysis, while the interface contacts of other side, between subunit A and C, might be involved in the binding for the substrate and the cofactor.

Several substrate analogs of 2,4-dienoyl-CoA reductase were synthesized as mechanistic probes for the purpose of understanding enzymatic reactions. The result showed that mDECR not only catalyze the reduction of substrates with chain lengths from 6-14 carbons with different double bond configurations, but also catalyze the conversion of the substrate analogs with the substitutions at C2 and C5 or with introduction of one more conjugated double bond between C6 and C7, however, with decreased catalytic efficiency. The effects of different substitutions supported a stepwise mechanism containing a dienolate intermediate. Our study also showed that 3-furan-2-yl-acrylyl-CoA is a competitive inhibitor of human mDECR.

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
ACS	Acyl-CoA Synthetase
ALA	Alpha-Linolei Acid
CD	Circular Dichroism
CHD	Coronary Heart Diease
DBP	D-Bifunctional Protein, or Peroxisomal Multifunctional 2-Enoyl-CoA Hydratase/(3*R*)-Hydroxyacyl-CoA Dehydrogenase also called MEF2
DECI	$\Delta^{3,5}$-$\Delta^{2,4}$-Dienoyl-CoA Isomerase
DECR	NADPH-Dependent 2,4-Dienoyl-CoA Reductase
ECH	2-Enoyl-CoA Hydratase
ECI	Δ^{3}-Δ^{2}-Enoyl-CoA Isomerase
ER	Endoplasmic Reticulum
FA	Fatty Acid
FCHL	Feruloyl-CoA Hydratase-lyase
FAD	Flavin Adenine Dinucleotide
$FADH_2$	Flavin Adenine Dinucleotide (reduced)
mFABP	membrane-associated Fatty Acid Binding Protein
cFABP	cytoplasmic Fatty Acid Binding Protein
H1/I	Hydratase1/Isomerase
HPLC	High Performance Liquid Chromatography
IC_{50}	lethal concentration to kill 50% of a population

K_M	Michaelis-Menten Constant
LA	Linoleic Acid
LCFA	Long Chain Fatty Acid
LBP	L-Bifunctional Protein, or Peroxisomal Multifunctional Δ^3-Δ^2-Enoyl-CoA Isomerase/2-Enoyl-CoA Hydratase1/(3*S*)-Hydroxyacyl-CoA Dehydrogenase, also called MEF1
MFE	Multi Functional Enzyme
MTF	Mitochondrial Trifunctional Enzyme
NAD^+	Nicotinamide Adenine Dinucleotide (oxidized)
NADH	Nicotinamide Adenine Dinucleotide (reduced)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced)
NMR	Nuclear magnetic resonance
PUFA	Poly Unsaturated Fatty Acid
SCOP	Structure Classification Of Protein
SCPx	Sterol Carrier Protein X
SDR	Short-chain Dehydrogenase/Reductase
SDS-PAGE	SDS-Polyacrylamide Gel Electrophoresis
V_{max}	Maximum velocity the enzyme can attain
VLCFA	Very Long Chain Fatty Acid

LIST OF FIGURES AND TABLES

LIST OF PUBLICATIONS DERIVED FROM THIS STUDY

Yu,W.; Chu,X.; Chen,G.; Li,D. Studies of human mitochondrial 2,4-dienoyl-CoA reductase. *Arch. Biochem. Biophys.* 2005; 434(1):195-200.

Alphey,M.S.; Yu,W.; Byres,E.; Li,D.; Hunter,W.N. Structure and reactivity of human mitochondrial 2,4-dienoyl-CoA reductase: enzyme-ligand interactions in a distinctive short-chain reductase active site. *J. Biol. Chem.* 2005; 280(4):3068-3077

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