Bioorganic Chemistry 47 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Comparative studies of Acyl-CoA dehydrogenases for monomethyl branched chain substrates in amino acid metabolism

Xiaojun Liu [b], Long Wu [b], Guisheng Deng [b], Gong Chen [b], Nan Li [b], Xiusheng Chu [b], Ding Li [a,*]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou University City, 132 Waihuan East Road, Guangzhou 510006, PR China
[b] Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, PR China

ARTICLE INFO

Article history:
Received 26 August 2012
Available online 29 December 2012

Keywords:
Branched-chain fatty acids
Branched-chain amino acids
Short/branched chain acyl-CoA dehydrogenase
Isovaleryl-CoA dehydrogenase
Isobutyryl-CoA dehydrogenase
Amino acid metabolism

ABSTRACT

Short/branched chain acyl-CoA dehydrogenase (SBCAD), isovaleryl-CoA dehydrogenase (IVD), and isobutyryl-CoA dehydrogenase (IBD) are involved in metabolism of isoleucine, leucine, and valine, respectively. These three enzymes all belong to acyl-CoA dehydrogenase (ACD) family, and catalyze the dehydrogenation of monomethyl branched-chain fatty acid (mmBCFA) thioester derivatives. In the present work, the catalytic properties of rat SBCAD, IVD, and IBD, including their substrate specificity, isomerase activity, and enzyme inhibition, were comparatively studied. Our results indicated that SBCAD has its catalytic properties relatively similar to those of straight-chain acyl-CoA dehydrogenases in terms of their isomerase activity and enzyme inhibition, while IVD and IBD are different. IVD has relatively broader substrate specificity than those of the other two enzymes in accommodating various substrate analogs. The present study increased our understanding for the metabolism of monomethyl branched-chain fatty acids (mmBCFAs) and branched-chain amino acids (BCAAs), which should also be useful for selective control of a particular reaction through the design of specific inhibitors.

1. Introduction

Three branched-chain amino acids (BCAAs), including isoleucine, leucine, and valine, have been found to play many essential roles in living organisms. For example, BCAAs are used by bacteria as major carbon and energy sources [1,2]. Lowered concentrations of BCAAs result in impaired growth and neurological problems [3]. BCAAs may be useful chemoprevention modality for colon cancer in obese people [4]. BCAAs can suppress insulin-resistance-based hepatocarcinogenesis [5]. BCAAs can modulate the function of proteins engaged in mRNA translation and the selection of specific mRNAs for translation through regulation of a signal transduction pathway, therefore, regulating process related to gene expression [6].

2-Methylbutyryl-CoA, isovaleryl-CoA (3-methylbutyryl-CoA), and isobutyryl-CoA are three short monomethyl branched-chain fatty acids (mmBCFAs) thioester derivatives, and derived from the catabolism of three BCAAs, isoleucine, leucine, and valine, respectively, as shown in Fig. 1. Their oxidations are catalyzed by short/branched chain acyl-CoA dehydrogenase (SBCAD, EC 1.3.99.3), isovaleryl-CoA dehydrogenase (IVD, EC 1.3.99.10), and isobutyryl-CoA dehydrogenase (IBD, EC 1.3.99.12), respectively. SBCAD, IVD, and IBD are mitochondrial homotetrameric flavoproteins containing one molecule of flavin adenine dinucleotide (FAD) per monomer, which all belong to acyl-CoA dehydrogenases (ACDs) family [7]. Deficiencies of these enzymes are important causes of human disease [8–14]. Although these three enzymes share similar sequences, catalytic mechanisms, and structural properties, the positions of their catalytic residues are not conserved in their primary sequences [15,16]. The crystal structure of human IVD has been solved at 2.6 Å resolution [17], and the crystal structure of human IBD with and without substrate has also been determined to 1.76 Å resolution [7]. The coordinates of the human SBCAD structure have been available for some time (pdb code: 2JIF), unfortunately its relevant paper has not been published. The overall fold of these enzymes has been found to be similar, and it is difficult to determine their catalytic differences based on their crystal structures. Besides, three-dimensional structures of the enzymes provide static pictures, while the enzymes are rather flexible in nature. Therefore, it would be interesting to have functional studies of these three enzymes comparatively. In the present paper, we report our comparative studies of these three enzymes through site-direc-

Abbreviations: BCAAs, branched-chain amino acids; BCFAs, branched chain fatty acids; DCPIP, 2,6-dichlorophenolindophenol; IBD, isobutyryl-CoA dehydrogenase; IPTG, isopropyl-β-D-thiogalactopyranoside; IVD, isovaleryl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; mmBCFAs, monomethyl branched-chain fatty acids; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PMS, phenazine methosulfate; SBCAD, short/branched chain acyl-CoA dehydrogenase; SDS, sodium dodecylsulfate; UV/Vis, ultraviolet-visible spectroscopy.

* Corresponding author. Fax: +86 20 3994 3058.
E-mail address: liding@mail.sysu.edu.cn (D. Li).

0045-2068/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bioorg.2012.12.001

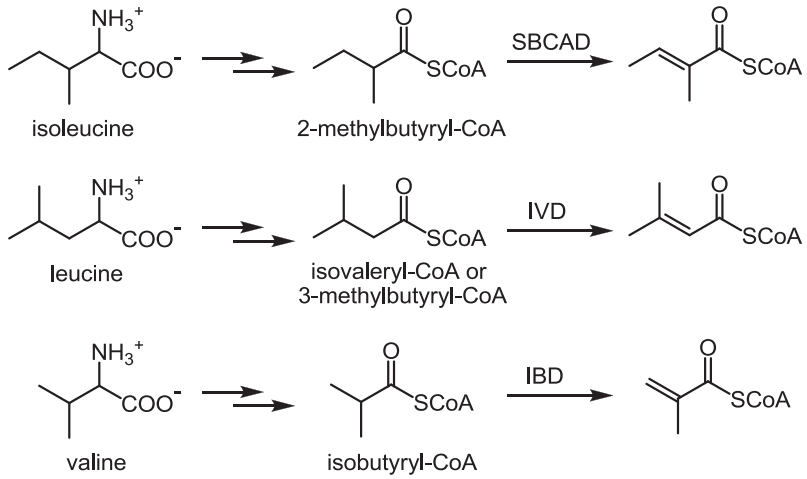

Fig. 1. Metabolism of branched-chain amino acids, isoleucine, leucine, and valine.

ted mutagenesis, kinetics, and incubation with various substrates and substrate analogs.

2. Materials and methods

2.1. Materials

A HiTrap chelating metal affinity column was purchased from Amersham Pharmacia Biotech. *Pfu* DNA polymerase, HB101 competent cells, *Escherichia coli* strain BL21(DE3) competent cells and agarose came from Invitrogen Life Technologies. The Plasmid Mini kit and synthesized oligonucleotides were obtained from Tech Dragon Company of Hong Kong. A gel extraction kit, T4 DNA ligase and restriction enzymes came from MBI Fermentas of Germany. Acyl-CoA thiolesters were prepared from the corresponding free acids and coenzyme A by using the mixed anhydride method [18] in our lab, and subsequently purified by using reverse-phase HPLC. All other reagents were of research grade or better and were obtained from commercial sources.

2.2. Cloning of the Gene of Rat SBCAD

A rat liver Quick-Clone cDNA library was purchased from Clontech (Palo Alto, CA). The gene of rat SBCAD was amplified by PCR using following primers. The sequence of the forward primer was 5′ G GAA TTC CAT ATG AAA TCC TC**C** CA**G** CC**G** GAA GCT CT**G** CT**G** AG**C** GTA AC**C** AA**C** AA**C** GC**T** CT**G** TG**C** TTC GCA CCT CTG CAG ACA TTT AC 3′, containing a Nde1 site (CAT ATG), and codons for amino acids 2–26 of rat liver SBCAD. In order to enhance expression, the nucleotide sequence of 12 codons at the 5′-end of the cDNA was altered to accommodate *E. coli* codon usage without altering the amino-acid coding sequence (highlighted with bold and underline). The sequence of the reverse primer was 5′ CTG CAG CTC GAG TCA GTA CTC TGC ATC GAT GTG C 3′, containing a Xho1 site (CTCGAG), a stop anticodon (TCA), and anticodons for the last seven amino acids of rat liver SBCAD. The PCR product was gel purified, double digested, and ligated into a pET28a expression vector resulting in the pET28a::SBCAD plasmid. The constructed pET28a::SBCAD plasmid was transformed into HB101 competent cells according to an electroporation transformation procedure (Bio-Rad) for screening purposes. The identified positive colony was grown in LB medium containing Ampicillin (50 mg/L), and the plasmid pET28a::SBCAD was isolated and transformed to *E. coli* strain BL21(DE3) competent cells for expression purposes. DNA sequencing of the cloned rat liver SBCAD gene was performed, and the inserted gene sequence was identified to be the same as that previously deposited in NCBI without any mutation.

2.3. Cloning of the Gene of Rat IVD

The gene of rat IVD was amplified by PCR using following primers. The sequence of the forward primer was 5′ GG CAA TTC CAT ATG CAC TC**T** ATG TT**T** CC**G** GTG GAT GAT GAT ATC AAC GG**T** CT**G** AAC GA**A** GA**A** CAG AA**A** CAG CT**G** CGT CAT AC**C** AT**C** TCT AAG TTC GTT CAA GAG AAC CTG 3′, containing a Nde1 site (CAT ATG), and codons for amino acids 2–32 of rat liver IVD. In order to enhance expression, the nucleotide sequence of 11 codons at the 5′-end of the cDNA was altered to accommodate *E. coli* codon usage without altering the amino-acid coding sequence (highlighted with bold and underline). The sequence of the reverse primer was 5′ CCG CAG AAG CTT CTA GCG GAA GTC TGC GTT GAA AG 3′, containing a Hind III site (AAGCTT), a stop anticodon (CTA), and anticodons for the last seven amino acids of rat liver IVD. The PCR product was gel purified, double digested, and ligated into a pET28a expression vector resulting in the pET28a::IVD plasmid. The constructed pET28a::IVD plasmid was identified, isolated and transformed to *E. coli* strain BL21(DE3) competent cells for expression purposes. DNA sequencing of the cloned rat liver IVD gene was performed, and the inserted gene sequence was identified to be the same as that previously deposited in NCBI without any mutation.

2.4. Cloning of the Gene of Rat IBD

For the cloning of rat isobutyryl-CoA dehydrogenase gene, the sequence of its forward primer was 5′ GCA CTA CAT ATG CTG GCT CAG ACC GAC CAC AGG 3′, containing a *Nde*I site (CATATG). The sequence of its reverse primer was 5′ CTG CAG CTC GAG CTA GTC CTG AAG CAG GCT CC 3′, containing a *Xho*I site (CTCGAG). The PCR product was gel purified, double digested by using *Nde*I and *Xho*I, and ligated into the pET28a expression vector digested by the same restriction enzymes, resulting in the plasmids pET28a::IBD. The positive colony with the expected gene was identified by single and double restriction digestion of the plasmid, followed with agarose gel analysis, and confirmed further by DNA sequencing.

2.5. Construction of mutants

A QuikChange mutagenesis kit (Stratagene) was applied for constructing the mutant expression plasmids. The plasmid pE-