近畿大学工学部研究報告 No39, 2005年, pp.15-18
Research Reports of the School of Engineering,
Kinki University No39, 2005, pp.15-18

Structural analysis of gene cluster encoding acetyl-CoA carboxylase from a deep-sea piezo- and psychrophilic bacterium, *Shewanella violacea* strain DSS12

Kaoru Nakasone

Acetyl-CoA carboxylase is key enzyme involved in the starting for fatty acid synthesis. In this study, we have cloned and sequenced gene cluster encoding acetyl-CoA carboxylase from a deep-sea piezo- and psychrophilic bacterium, *Shewanella violacea* strain DSS12. Sequence and structural analyses were done to understand structural-function relationships for adapting under high pressure and low temperature conditions. The cloned fragment contained two open reading frames, designated the *accB* and *accA* genes, capable of encoding a 538-amino-acid protein of 58.1 kDa and a 573-amino-acid protein of 61.5 kDa, respectively. The protein (AccA) encoded by the *accA* gene was strikingly similar to biotin carboxylase subunits of acetyl-CoA and propionyl-CoA carboxylases and of pyruvate carboxylase. The putative motifs for ATP binding, CO_2 fixation, and biotin binding were found in AccA. The *aceB* gene was located upstream of the *accA* gene, and they formed a two-gene operon. The protein (AccB) encoded by the *aceB* gene showed high degrees of sequence similarity with carboxyltransferase subunits of acetyl-CoA and propionyl-CoA carboxylases and of methylmalonyl-CoA decarboxylase. Carboxybiotin-binding and acyl-CoA-binding domains, which are conserved in several carboxyltrausferase subunits of acyl-CoA carboxylases, were found in AccB.

Key words *Shewanella violacea*, acetyl-CoA carboxylase, prokaryote, eukaryote, evolution

Introduction

Acetyl-CoA carboxylase catalyzes the ATP-dependent carboxylation of acetyl-CoA to yield malonyl-CoA. In *Escherichia coli*, the acetyl-CoA carboxylases consist of three functional units: carboxyltransferase, biotin carboxyl carrier protein and biotin carboxylase[1]. The *E. coli* acetyl-CoA carboxylase is the only biotinylated protein in *E. coli* [2] and the enzyme does not catalyze a reaction analogous to that of propionyl-CoA carboxylase. In contrast to bacterial enzymes, eukaryotic acetyl-CoA carboxylases are large enzymes and contain all components in a single protein. Propionyl-CoA carboxylase forms methylmalonyl-CoA from propionyl CoA by CO, fixation. Methylmalonyl-CoA serves as a precursor for the synthesis of branched -chain fatty acids. All propionyl-CoA carboxylases from prokaryotes and eukaryotes consist of two nonidentical subunits, biotin carboxylase and carboxyltransferase. The bacterial acyl-CoA carboxylases isolated from *Mycobacterium pheli*, *Mycobacterium smegnzatis*, and Streptomyces erythreus show maximal rates of carboxylation with propionyl-CoA, but the enzymes are also able to carboxylate acetyl-CoA well [3]. In several bacteria, a single enzyme with dual-substrate specificity catalyzes the carboxylation of both acetyl- and propionyl-CoA.

Shewanella violacea strain DSS12 is a psychrophilic and facultatively piezophilic bacterium which was isolated from the mud of the Ryukyu Trench (5,110m depth) collected by the manned-submersible SHINKAI 6500[4, 5]. This bacterium displays optimal growth at a temperature of 8 C. *S. violacea* is one of the well-investigated piezophiles [6], and genome analysis of this organism is underway. Especially, the organism can be an excellent source for the study of bacterial adaptation to the environment of high hydrostatic pressure because it showed significant growth both under a condition of high pressure and atmospheric pressure. As an evidence of piezo-adaptation, a pressure-regulated promoter was found in this bacterium [7]. We attempted to clone the acetyl-CoA carboxylase gene from *S. violacea* using appropriate oligonucleotide probes designed from the conserved sequences in the acetyl-CoA carboxylases.

Here, we report the cloning and sequencing of the *accA* and *accB* genes encoding acetyl-CoA carboxylase from *S. violacea* and we discuss the structure and function of this enzyme.

近畿大学工学部生物化学工学科

Department of Biotechnology and Chemistry,
School of Engineering, Kinki University

Materials and Methods

Bacterial strains and culture conditions

S. violacea DSS12 was cultured as described previously [4]. Marine Broth 2216 was used as the medium. The seeded medium was placed into sterilized soft plastic packages, and the packages were tightly packed without gas phase. Cultivation was performed in pressure vessels at 8 C under various pressures for 2 days. Under this growth condition, no more O_2 was dissolved to the medium by pressurization, and absolute O_2 concentration in the medium was not changed with pressurization. *S. violacea* cannot grow by fermentation at least in this time scale. Therefore, the cells of the organism could utilize O_2 which was solubilized to the medium initially and were grown under the microaerobic conditions despite of its pressure. For spectrophotometric analyses, large-scale cultivation of the organism under a high pressure and microaerobic condition was performed with DEEP-BATH system in JAMSTEC. This system is also capable for cultivation of the organism under high pressure without dissolving of excess O_2, because culture vessel contains seeded medium without gas phase. Also, for spectrophotometric analyses, cultivation of the organism under atmospheric pressure with various conditions was performed in 500-ml shaking flask. *E. coli* was cultivated on LB medium containing appropriate antibiotics if needed.

Isolation of acc gene cluster from S. violacea

An *S. violacea* genomic DNA library was prepared by partially digesting chromosomal DNA with *Sau*3AI, ligating the DNA with *Bam*HI cleaved lDASHII phage vector, and then packaging the DNA into phage particles. For the detection of the acetyl-CoA carboxylase gene of *S. violacea*, oligonucleotides were designed as DNA probes for hybridization experiments. The oligonucleotides were labeled with digoxygenin (DIG)-11-dUTP by using an oligonucleotide tailing kit. several positive phage clones were screened by plaque hybridization with an oligonucleotide probe (ACC1). The sequence of ACC1 is 5'-(G/C)GCGATCPC(G/C)CC(G/C)CGGTTCG-3' (oligo-1); it was designed on the basis of the consensus sequence (ANRGEIA) of acetyl-CoA carboxylases of *E. coli* and *Anabaena* sp. strain PCC 7120 [8].

DNA sequencing

Nucleotide sequences were determined by the dideoxynucleotide chain termination method [9] using a model ABI 310 DNA sequencer. Both directional strands were completely analyzed by overlapping at every junction.

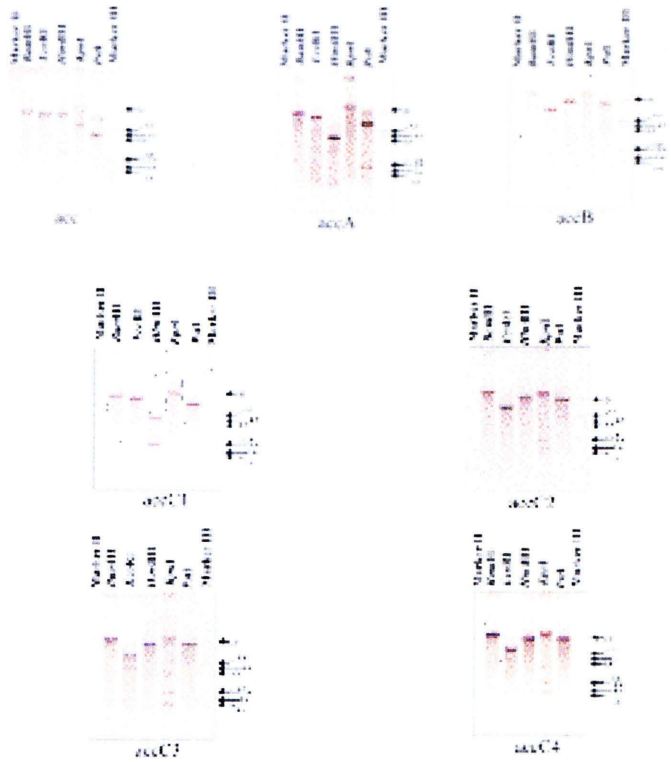

Fig. 1 Southern hybridization experiments with digoxygenin as probes

Results and discussion

Isolation of the genes and its structural analyses

Several positive clones containing acetyl-CoA carboxylase related genes were isolated with plaque hybridization experiments. Subcloning and sequencing of the isolated clones revealed that structural genes for *accA*, *accB*, *accC* and *accD* of acetyl-CoA carboxylase were encoded on the genome of *S. violacea*. In order to distribution and copy numbers on the genome of the genes, Southern hybridization experiments with digoxygenin as probes, were carried out. Figure 1 showed the detection of the genes (*accA*, *accB*, *accC* and *accD*) for acetyl-CoA carboxylase, suggesting the distribution of the genes for, not only prokaryotic types for subunit structures, but also eukaryotic type for multifunctional subunit structure. This observation is the first example in prokaryotic studies, and the results show that horizontal transfer of the gene(s) from some eukaryotes in the evolution of the strain. The amino acid sequence of the gene of eukaryotic type by BLAST searches, is very similar to yeast, for example (Table 1). Physiological significances in the gene transfer from eukaryote to the strain, however, remain to be elucidated. For the biochemical functions of the products, complementary experiments with several mutants such as *Escherichia coli* and *Saccharomyces cerevisiae*, will be required for future study.

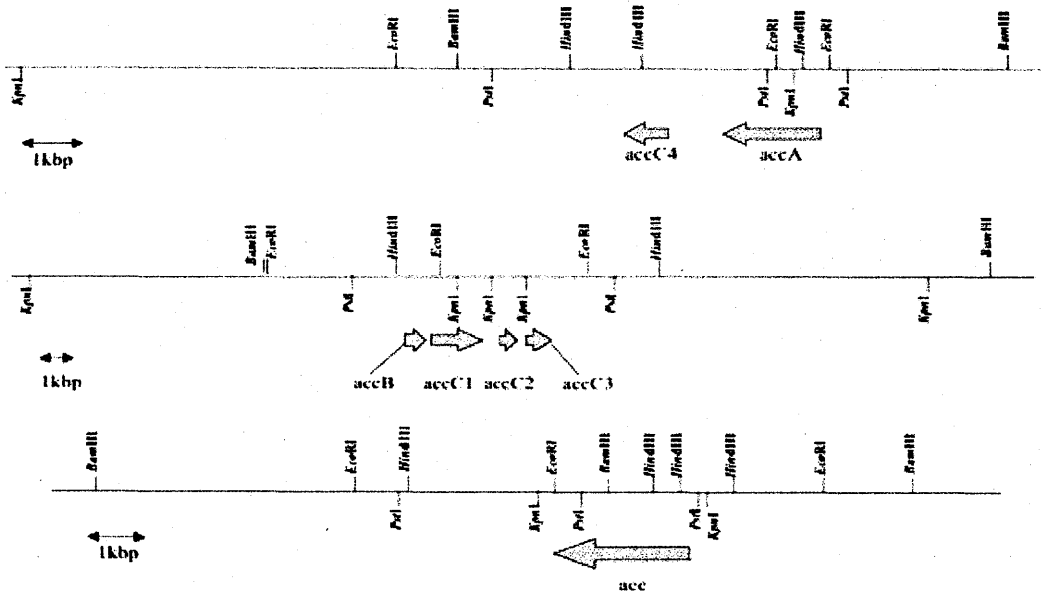

Fig. 2 Distribution of the genes for acetyl-CoA carboxylase (*accA*, *accB*, *accC*, *accD*)

Table 1 BLAST search of the cloned genes (*accA*, *accB*, *accC*, *accD*)

	species	accession No	product	nt	aa
	Shewanella amazonensis	Q8EFS0	3-methylcrotonyl CoA carboxylase, beta subunit	1668	535
	Idiomarina loihiensis	Q5QW27	3-methylcrotonyl CoA carboxylase, beta subunit	1668	535
accA	Vibrio parahaemolyticus	Q87H39	Putative acyl-CoA carboxyltransferase beta chain	1668	535
	Ralstonia solanacearum	Q8Y2R6	Putative propionyl-CoA carboxylase, beta subunit	1668	535
	Bordetella bronchiseptica	Q7WDP6	Carboxyltransferase subunit of acetyl-CoA carboxylase	1668	535
	Photobacterium profundum	Q6LLV9	acetyl-CoA carboxylase, biotin carboxyl carrier protein	459	152
accB	Vibrio parahaemolyticus	Q87KU5	acetyl-CoA carboxylase, biotin carboxyl carrier protein	459	152
	Vibrio vulnificus	Q7MEG1	acetyl-CoA carboxylase, biotin carboxyl carrier protein	465	154
	Idiomarina loihiensis	Q5QVU0	biotin carboxylase	1350	449
accC1	Yersinia pestis	AD0445	biotin carboxylase	1350	449
	Escherichia coli	AACC_ECOLI	biotin carboxylase	1350	449
	Yersinia pestis	AD0445	biotin carboxylase	1350	449
accC2	Photobacterium profundum	Q7V928	biotin carboxylase	1350	449
	Erwinia carotovora	Q6DAJ3	biotin carboxylase	1344	447
	Yersinia pestis	AD0445	biotin carboxylase	1350	449
accC3	Photobacterium profundum	Q7V928	biotin carboxylase	1350	449
	Erwinia carotovora	Q6DAJ3	biotin carboxylase	1344	447
	Shewanella amazonensis	Q8EFS2	biotin carboxylase	2085	694
	Idiomarina loihiensis	Q5QW25	3-methylcrotonyl-CoA carboxylase alpha chain	1971	656
accC4	Ralstonia solanacearum	Q8Y2S6	Probable acyl-CoA carboxylase alpha chain	2139	712
	Xanthomonas axonopodis	Q6GMU7	MGC81895 protein	2151	716
	Pseudomonas aeruginosa	AR1595	probable acyl-CoA carboxylase alpha chain	1988	662

Evolution of acetyl-CoA carboxylase

The acetyl-CoA carboxylases can be divided into two basic types, a bacterial type and a eukaryotic type, by their structure. The bacterial type contains four dissociated proteins, the biotin carboxylase, the biotin carboxyl carrier protein, and two carboxyltransferase subunits (α and β), organized into three functional domains (Fig. 2). The α and b subunits of the carboxyltransferase component of *E. coli* have acyl-CoA-binding and carboxybiotin-binding domains, respectively. The genes (*accA* and *accD*) encoding the two carboxyltransferase subunits are located almost directly opposite each other in the *E. coli* chromosome. In the eukaryotic type, these proteins are part of a single multifunctional polypeptide derived from the expression of a single gene. From the amino terminus, the biotin carboxylase component, biotin-binding site, carboxybiotin-binding site, and acyl-CoA-binding domain are distributed in this order along the eukaryotic acetyl-CoA carboxylase. The results of this study indicated that *S. violacea* AccA may be a biotinylated biotin carboxylase subunit of acetyl-CoA carboxylase. For functional analyses of the proteins, expression vectors harboring these genes are being constructed.

References

1) Alberts, A., Acetyl-CoA carboxylase, 37-82. In P.D. Boyer (ed.), The enzymes, Academic Press, Inc., New York (1976)

2) Fall, R. R., A. W. Alberts, P. R. Vagelos, Analysis of bacterial biotin proteins, Biochim. Biophys. Acta, 379, 496-503 (1975)

3) Erfle, J. D., Acetyl-CoA and propionyl-CoA carboxylation by Mycobacterium pheli; partial purification and some properties of the enzyme, Biochim. Biophys. Acta, 316, 143-155 (1973)

4) Kato C, Sato T, Horikoshi K, Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. Biodiv Conserv 4:1–9 (1995)

5) Nogi Y, Kato C, Horikoshi K, Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov. Arch Microbiol 170:331–338 (1998)

6) Nakasone K, Ikegami A, Kawano H, Kato C, Usami R, Horikoshi K, Transcriptional regulation under pressure conditions by RNA polymerase s^{54} factor with a two-component regulatory system in *Shewanella violacea*. Extremophiles 6:89–95 (2002)

7) Kato C, Smorawinska M, Sato T, Horikoshi K, Analysis of a pressure-regulated operon from the barophilic bacterium strain DB6705. Biosci Biotechnol Biochem 60:166–168 (1996)

8) Gornicki, P. I. Genes for two subunits of acetyl coenzyme A carboxylase of Anabaena sp. strain PCC 7120: biotin carboxylase and biotin carboxyl carrier protein, J Bacteriol., 175, 5268-5272 (1993)

9) Sanger, F., Nicklen, S., A. Coulson, DNA sequencing with chain terminating inhibitors, Proc. Natl. Acad. Sci. USA, 74, 5463-5467 (1977)