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Acetyl-CoA carboxylase from *Escherichia coli*: Gene organization and nucleotide sequence of the biotin carboxylase subunit

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ABSTRACT **Biotin carboxylase [biotin-carboxyl-carrier-protein:carbon-dioxide ligase (ADP-forming), EC 6.3.4.14] is the enzyme mediating the first step of the acetyl-CoA carboxylase [acetyl-CoA:carbon-dioxide ligase (ADP-forming), EC 6.4.1.2] reaction. We screened an *Escherichia coli* DNA library and a DNA fragment carrying the biotin carboxylase gene *fabG*, and its flanking regions were cloned. The gene for biotin carboxyl carrier protein was found 13 base pairs upstream of the *fabG* gene. Nucleotide sequencing of the recombinant plasmids revealed that the *fabG* codes for a 449-amino acid residue protein with a calculated molecular weight of 49,320, a value in good agreement with that of 51,000 determined by SDS/polyacrylamide gel electrophoresis of the purified enzyme. The deduced amino acid sequence of biotin carboxylase is also consistent with the partial amino acid sequence determined by Edman degradation. The primary structure of this enzyme exhibits a high homology with those of other biotin-dependent enzymes and carbamoyl-phosphate synthetase [carbon-dioxide:L-glutamine amido-ligase (ADP-forming, carbamate-phosphorylating), EC 6.3.5.5]; therefore, all these enzymes probably function through the same mechanism of reaction.**

Biotin-dependent enzymes mediate carboxylation, transcarboxylation, and decarboxylation of various compounds (1). ATP-requiring carboxylases constitute one class of enzymes and comprise those of acetyl-CoA carboxylase [ACC; acetyl-CoA:carbon-dioxide ligase (ADP-forming), EC 6.4.1.2] and pyruvate carboxylase [PC; pyruvate:carbon-dioxide ligase (ADP-forming), EC 6.4.1.1]. The carboxylation reaction mediated by these carboxylases takes place in two discrete steps with bicarbonate as the carboxyl donor (2). In the first partial reaction enzyme-bound (E) biotin is carboxylated on its ureido nitrogen (reaction 1) and the carboxyl group is transferred to an acceptor substrate (RH) such as acetyl-CoA and pyruvate in the second partial reaction (reaction 2).

$$\text{E-biotin} + HCO_3^- + \text{ATP} \rightleftharpoons \text{E-biotin-}CO_2^- + \text{ADP} + P_i \quad [1]$$

$$\text{E-biotin-}CO_2^- + \text{RH} \rightleftharpoons \text{E-biotin} + \text{R-COOH} \quad [2]$$

The first partial reaction is unique for ATP-requiring carboxylases and has been the target of intensive investigation (3). Detailed studies on mechanisms have been hampered in part because the two partial reactions are inseparable in most cases. ACC from *Escherichia coli* is one exception. This enzyme is composed of three different subunits—biotin carboxyl carrier protein (BCCP), biotin carboxylase [BC; biotin-carboxyl-carrier-protein:carbon-dioxide ligase (ADP-forming), EC 6.3.4.14], and carboxyl transferase (CT)—that readily dissociate with retention of biochemical activity (4). The BC subunit, which catalyzes the first partial reaction, was purified to apparent homogeneity (5) and has been used for studies on reaction mechanisms (6, 7). Structural data have heretofore not been available. We describe here the organization of the genes for BCCP and BC and the amino acid sequence of the latter subunit.‖

MATERIALS AND METHODS

Materials. Restriction endonucleases, T4 DNA ligase, pUC18, dideoxynucleotides, and two types of sequencing primers were obtained from Toyobo (Osaka). Sequenase was the product of United States Biochemical. [α-^{32}P]dCTP and [γ-^{32}P]ATP were obtained from ICN or Amersham. *E. coli* DNA library in λ phage is that of Kohara *et al.* (8). BC was purified as described (5, 9).

General Methods. Bacteria were grown in Luria–Bertani broth. Plasmids were propagated in *E. coli* DH5α [*supE44*, *ΔlacU169* (*φ* 80*lacZ*ΔM15)*hsdR17,recA1,endA1,gyrA96,thi-1,relA1*]. All gene manipulations were carried out by standard procedures (10).

Protein Sequencing. BC (250 μg) was pyridylethylated and then digested with *Achromobacter* proteinase I in a 50:1 ratio, and the generated peptides were separated by reversed-phase HPLC (11). Some of the fractions as well as the native enzyme were subjected to amino acid sequencing on an Applied Biosystems 470A protein sequenator connected with a 120A HPLC system (11).

Nucleotide Synthesis. Based on the peptide sequences of BC (Gly-Asp-Asp-Met-Asp-Lys) and BCCP (Met-Lys-Met-Met-Asn-Gln-Ile; ref. 12), the following two nucleotides were synthesized on an Applied Biosystems 381A DNA synthesizer: 5′-TT(A/G)TCCAT(A/G)TC(A/G)TCNCC-3′ and 5′-AT(T/C)TG(A/G)TTCATCAT(T/C)TTCAT-3′, where N is any nucleotide. They were end-labeled with ^{32}P and termed BC or BCCP probe, respectively.

Southern Blotting. Electrophoresis of restriction enzyme digests was on a 0.7% agarose gel. DNA fragments were separated and transferred onto a nylon membrane (DuPont) on an LKB Vacuogene and then allowed to hybridize with either the BC or BCCP probe. The filters were washed at 28°C for the BC and at 37°C for the BCCP probe and were then subjected to autoradiography.

DNA Sequencing. The DNA sequence of recombinant plasmids was determined by the dideoxynucleotide chain-termination method using Sequenase and two types of prim-

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Abbreviations: ACC, acetyl-CoA carboxylase; BC, biotin carboxylase; BCCP, biotin carboxyl carrier protein; CT, carboxyl transferase; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; CPS, carbamoyl-phosphate synthetase.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M79446).

ers as described elsewhere (13). All sequences were obtained for both strands of the DNA.

RESULTS

Cloning and Location of the BC Gene. A purified preparation of BC was digested with *Achromobacter* proteinase I and the resulting peptides, together with the native protein, were analyzed with an automatic protein sequenator. Based on a part of the sequences (Gly-Asp-Asp-Met-Asp-Lys) an oligonucleotide probe was synthesized.

The *E. coli* DNA library of Kohara *et al.* (8) was screened with the probe and a single clone that gave a strong signal was isolated. This clone, designated 6G3, was located between 70 and 90 min of the *E. coli* chromosome, but the exact location was not elucidated because of the complexity of the region (8). The phage clone was amplified and digested with various restriction endonucleases to construct a restriction map. This map, shown in Fig. 1*A*, is consistent for the most part with that of Kohara *et al.* (8) for this region. A 3.6-kilobase (kb) *Kpn* I fragment (K_1), which hybridized with the BC probe on Southern blotting, was cloned into pUC18 to yield plasmid pAK1.

Nucleotide Sequence of the BC Gene. The nucleotide sequence of pAK1 harboring the BC gene was determined by the dideoxynucleotide chain-termination method according to the strategy shown in Fig. 1*B* and the result is shown in Fig. 2.

The amino acid sequences of peptide fragments derived from BC were aligned with the amino acid sequence deduced from the nucleotide sequence of the DNA. These sequences cover nearly half of the entire sequence and match well those predicted from the DNA sequence (positions 1–1347). The open reading frame is 1347 base pairs (bp) long and would code for a protein of 449 amino acids with a calculated molecular weight of 49,320. This value is in good agreement with the molecular weight of 51,000 determined for the BC enzyme by SDS/polyacrylamide gel electrophoresis (5).

Organization of the BC and the BCCP Genes. There is an open reading frame 13 bp upstream of the BC gene and the nucleotide sequence of this region corresponds to that assigned to the BCCP gene (14). To confirm that the BCCP gene is indeed located here, an oligonucleotide probe for BCCP was prepared according to a part of the amino acid sequence for BCCP (Met-Lys-Met-Met-Asn-Gln-Ile; ref. 12). This probe gave a strong signal with clone 6G3, which carries the BC gene. The BCCP gene was assigned to 72 min on the chromosome (15). Our present finding supports this location.

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GGTACCGTGAAAGCAATTCTGGTCGAAAGTGGACAACCGGTAGAATTTGACGAGCCGCTGGTCGTCATCGAGTAACGAGGCGAAC   -1
ATGCTGGATAAAATTGTTATTGCCAACCGCGGCGAGATTGCATTGCGTATTCTTCGTGCCTGTAAAGAACTGGGCATCAAGACTGTCGCT   90
 M  L  D  K  I  V  I  A  N  R  G  E  I  A  L  R  I  L  R  A  C  K  E  L  G  I  K  T  V  A    30
GTGCACTCCAGCGCGGATCGCGATCTAAAACACGTATTACTGGCAGATGAAACGGTCTGTATTGGCCCTGCTCCGTCAGTAAAAAGTTAT   180
 V  H  S  S  A  D  R  D  L  K  H  V  L  L  A  D  E  T  V  C  I  G  P  A  P  S  V  K  S  Y    60
CTGAACATCCCGGCAATCATCAGCGCCGCTGAAATCACCGGGCGCAGTAGCAATCCATCCGGGTTACGGCTTCCTCTCCGAGAACGCCAAC   270
 L  N  I  P  A  I  I  S  A  A  E  I  T  G  A  V  A  I  H  P  G  Y  G  F  L  S  E  N  A  N    90
TTTGCCGAGCAGGTTGAACGCTCCGGCTTTATCTTCATTGGCCCGAAAGCAGAAACCATTCGCCTGATGGGCGACAAAGTATCCGCAATC   360
 F  A  E  Q  V  E  R  S  G  F  I  F  I  G  P  K  A  E  T  I  R  L  M  G  D  K  V  S  A  I   120
GCGGCGATGAAAAAAGCGGGCGTCCCTTGCGTACCGGGTTCTGACGGCCCGCTGGGCGACGATATGGATAAAAACCGTGCCATTGCTAAA   450
 A  A  M  K  K  A  G  V  P  C  V  P  G  S  D  G  P  L  G  D  D  M  D  K  N  R  A  I  A  K   150
CGCATTGGTTATCCGGTGATTATCAAAGCCTCCGGCGGCGGCGGCGGTCGCGGTATGCGCGTAGTGCGCGGCGACGCTGAACTGGCACAA   540
 R  I  G  Y  P  V  I  I  K  A  S  G  G  G  G  G  R  G  M  R  V  V  R  G  D  A  E  L  A  Q   180
TCCATCTCCATGACCGTGCGGAAGCGAAAGCTGCTTTCAGCAACGATATGGTTTACATGGAGAAATACCTGGAAAATCCTCGCCACGTC   630
 S  I  S  M  T  R  A  E  A  K  A  A  F  S  N  D  M  V  Y  M  E  K  Y  L  E  N  P  R  H  V   210
GAGATTCAGGTACTGGCTGACGGTCAGGGCAACGCTATCTATCTGGCGGAACGTGACTGCTCCATGCAACGCCGCCACCAGAAAGTGGTC   720
 E  I  Q  V  L  A  D  G  Q  G  N  A  I  Y  L  A  E  R  D  C  S  M  Q  R  R  H  Q  K  V  V   240
GAAGAAGCGCCAGCACCGGGCATTACCCCGGAACTGCGTCGCTACATCGGCGAACGTTGCGCTAAAGCGTGTGTTGATATCGGCTATCGC   810
 E  E  A  P  A  P  G  I  T  P  E  L  R  R  Y  I  G  E  R  C  A  K  A  C  V  D  I  G  Y  R   270
GGTGCAGGTACTTTCGAGTTCCTGTTCGAAAACGGCGAGTTCTATTTCATCGAAATGAACACCCGTATTCAGGTAGAACACCCGGTTACA   900
 G  A  G  T  F  E  F  L  F  E  N  G  E  F  Y  F  I  E  M  N  T  R  I  Q  V  E  H  P  V  T   300
GAAATGATCACCGGCGTTGACCTGATCAAAGAACAGCTGCGTATCGCTGCCGGTCAACCGCTGTCGATCAAGCAAGAAGAAGTTCACGTT   990
 E  M  I  T  G  V  D  L  I  K  E  Q  L  R  I  A  A  G  Q  P  L  S  I  K  Q  E  E  V  H  V   330
CGCGGCCATGCGGTGGAATGTCGTATCAACGCCGAAGATCCGAACACCTTCCTGCCAAGTCCGGGCAAAATCACCCGTTTCCACGCACCT  1080
 R  G  H  A  V  E  C  R  I  N  A  E  D  P  N  T  F  L  P  S  P  G  K  I  T  R  F  H  A  P   360
GGCGGTTTTGGCGTACGTTGGGAGTCTCATATCTACGCGGGCTACACCGTACCGCCGTACTATGACTCAATGATCGGTAAGCTGATTTGC  1170
 G  G  F  G  V  R  W  E  S  H  I  Y  A  G  Y  T  V  P  P  Y  Y  D  S  M  I  G  K  L  I  C   390
TACGGTGAAAACCGTGACGTGGCGATTGCCGCATGAAGAATGCGCTGCAGGAGCTGATCATCGACGGTATCAAAACCAACGTTGATCTG  1260
 Y  G  E  N  R  D  V  A  I  A  R  M  K  N  A  L  Q  E  L  I  I  D  G  I  K  T  N  V  D  L   420
CAGATCCGCATCATGAATGACGAGAACTTCCAGCATGGTGGCACTAACATCCACTATCTGGAGAAAAAACTCGGTCTTCAGGAAAAATAA  1350
 Q  I  R  I  M  N  D  E  N  F  Q  H  G  G  T  N  I  H  Y  L  E  K  K  L  G  L  Q  E  K  *   449
GACTGCTAAAGCGTCAAAAGGCCGGATTTTCCCTTTTTTATTACTGGGGATCGACAACCCCCATAAGGTACAATCCCCGCTTTCTTCACC  1440
CATCAGGGACAAAAAATGGACACTCGTTTTGTTCAGGCCCATAAAGAGGCGCGCTGGGCTGGGCTGGGCCTTTTGTATCTGGCAGTTTG  1530
GTTAGTAGCCGCTTACTTATCTGGCGTTGCCCCGGTTTTACCGGCTTTCCGCGCTGGTTTGAGATGGCCTGCATCCTGACGCCGCTGCTG  1620
TTTATTGGACTGTGCTGGGCGATGGTGAAATTTATCTATCGCGAT                                              1665
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FIG. 2. Nucleotide sequence and deduced amino acid sequence for *E. coli* BC. The nucleotide sequence represents the region from the middle *Kpn* I site through the 3′ side *Eco*RV site in Fig. 1. Amino acid sequences determined by Edman degradation are underlined. The nucleotide sequence used as the BC hybridization probe is overlined.

On Southern blotting, the BCCP probe hybridized with a 1.2-kb *Kpn* I fragment (K_2), derived from clone 6G3. A 1.6-kb *Pvu* II fragment (P_1), which overlaps part of the K_1 and K_2 fragments, gave a positive signal with both the BC and BCCP probes. This clearly shows that the genes for BC and BCCP are in close proximity within this region (Fig. 1*A*).

DISCUSSION

ACC plays an indispensable role in fatty acid biosynthesis in all living organisms. Unlike multifunctional eukaryotic enzymes, the bacterial ACC is composed of subunits that readily dissociate with retention of specific biochemical

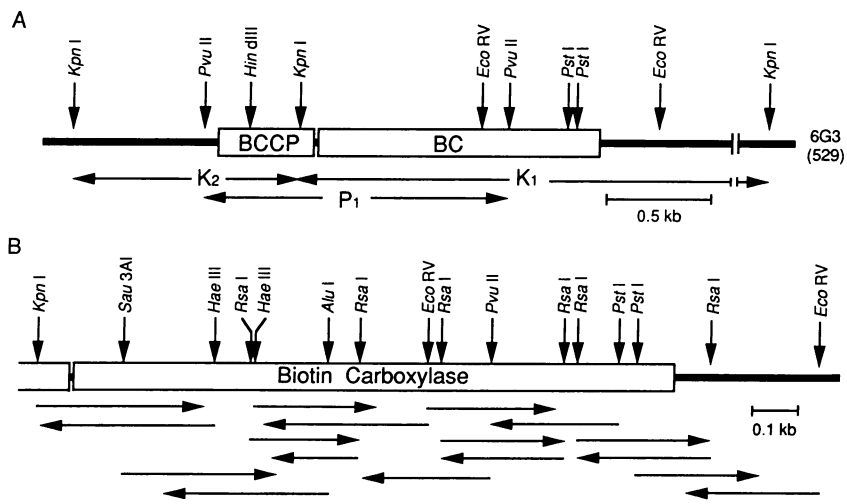

FIG. 1. Restriction enzyme map (*A*) and sequencing strategy (*B*) for the gene of *E. coli* BC. Open boxes represent coding regions for the BCCP and BC genes. Arrows indicate the direction and extent of sequencing.

activities (4, 5). In this respect, *E. coli* ACC offers advantages for examining the mechanism and regulation of fatty acid biosynthesis. As knowledge of gene structures is a prerequisite for achieving this goal, we cloned and characterized the genes for *E. coli* ACC.

The gene for one of the subunits, BCCP, was located at 72 min on the *E. coli* chromosome (15) and was recently sequenced by Muramatsu and Mizuno (14). We cloned and sequenced the gene for another subunit, BC. This gene, named *fabG*, is located immediately downstream of the BCCP gene and the two appear to form an operon. The gene for the third subunit, CT, remains to be identified because of the lack of protein sequence data. It is conceivable that the CT gene is located in the vicinity of the BCCP and BC genes. With this in mind, we sequenced the regions immediately upstream and downstream of the genes but were unable to find a possible candidate.

BC exhibits a high homology with other biotin-dependent enzymes such as chicken (16) and rat ACC (17), rat propionyl-CoA carboxylase [PCC; propionyl-CoA:carbon dioxide ligase (ADP-forming), EC 6.4.1.3] (18), and yeast PC (19) (Fig. 3). Thus, BC and chicken ACC share identical amino acids at 167 sites of the 449-residue protein for BC (36.5% identity), while BC and the α subunit of rat PCC are identical at 208 sites over the same region (46.1% identity). Analogously, BC and yeast PC are 45.3% identical and homology increases to 60.2%, 66.4%, and 66.2%, respectively, if conservative substitutions are taken into account (for definition of amino acid similarities, refer to Fig. 3). Most notable is the finding that *E. coli* BC is more homologous with rat PCC and yeast PC than with animal ACC. By aligning the protein sequences of *E. coli* BC and other biotin-dependent carboxylases, the BC domain of the latter enzymes can be defined with certainty. Thus, the BC domain of chicken ACC may initiate at about residue 118 and terminate at about residue 620 (Fig. 3).

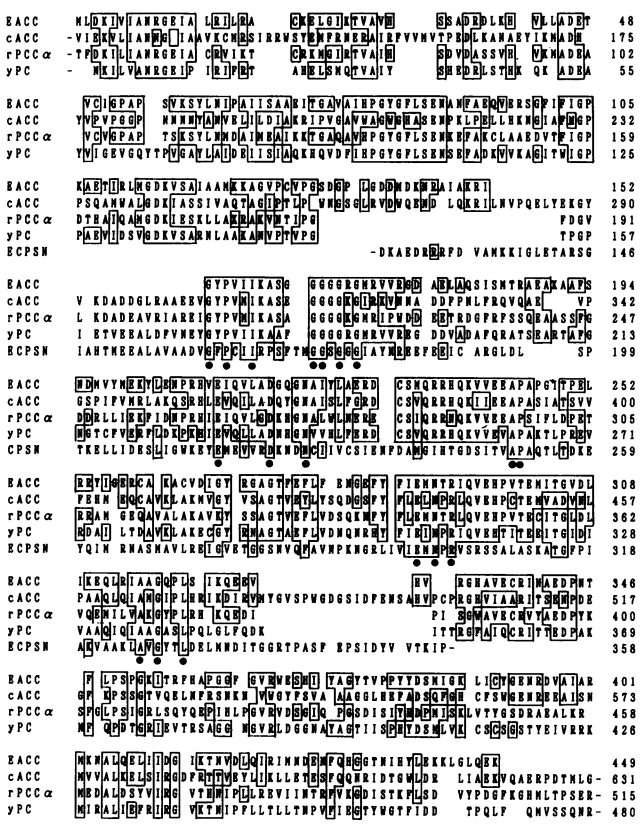

FIG. 3. Comparison of the amino acid sequences of biotin-dependent and related enzymes in their BC domain. EACC, *E. coli* ACC; cACC, chicken ACC; rPCCα, α subunit of rat PCC; yPC, yeast PC; ECPSN, N-terminal half of *E. coli* CPS. Identical amino acids are boxed and strictly conserved residues are marked by dots. As for conservative substitutions (see text), the following sets of amino acids are regarded as being homologous (20): cysteine, serine, threonine, proline, alanine, and glycine; asparagine, aspartic acid, glutamic acid, and glutamine; histidine, arginine, and lysine; methionine, isoleucine, leucine, and valine; and phenylalanine, tryptophan, and tyrosine.

E. coli BC as well as animal ACC, PCC, and yeast PC exhibit considerable homology with carbamoyl-phosphate synthetase [CPS; carbon-dioxide:L-glutamine amido-ligase (ADP-forming, carbamate-phosphorylating), EC 6.3.5.5] from various sources (21–24). For example, BC and *E. coli* CPS are 22.9% identical and 48.0% similar over the 153–328 region for BC. The corresponding values for BC vs. chicken ACC are 46.9% and 68.0%, respectively. Given the similarity of the reactions they catalyze (reactions **1** and **3**),

$$\text{Glutamine} + HCO_3^- + 2\ \text{ATP} \rightleftharpoons \text{carbamoyl phosphate} + \text{glutamic acid} + 2\ \text{ADP} + P_i \quad [3]$$

this may suggest that all these enzymes function through an identical or similar mechanism. BC is capable of generating ATP from carbamoyl phosphate and ADP (25); therefore, it seems reasonable to assume that essential amino acid residues are conserved in the enzymes in question. Eighteen amino acid residues are strictly conserved in the four carboxylases and *E. coli* CPS as marked by dots in Fig. 3. Some of the conserved residues are clustered and two of these clustered regions attracted our attention. One region encompasses residues 163–168 of BC with the sequence Gly-Gly-Gly-Gly-Arg-Gly. Although the stringency of homology breaks and the second glycine from the left is the sole conserved amino acid, if nonbacterial CPSs are included, this region appears to be an ATP-binding site for BC. Nucleotide-binding proteins share consensus sequences such as Gly-Xaa-Gly-Xaa-Xaa-Gly or Gly-Xaa-Xaa-Gly-Xaa-Gly (26, 27). The above region matches these motifs perfectly and probably constitutes part of the ATP-binding site of BC.

Another conserved region is located at 288–292 of BC, as Glu-Met-Asn-Pro-Arg. It is noteworthy that three of five residues are conserved and that this conservation extends to nonbacterial CPSs. This region could be a strong candidate for the catalytic site of biotin-dependent carboxylases and CPS, since functional residues such as glutamic acid and arginine are present there. This notion is based on the observation that treatment of CPS with dicarbonyl reagents such as phenylglyoxal modified the arginine residues, thereby abolishing the enzymatic activity of CPS (28). Whether or not Arg-292 of BC is essential for catalysis of this enzyme warrants further study by site-directed mutagenesis.

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