be important in determining the cellulsar distribution of these class 1 antigens. We are also presently studying these possibilities.

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Primary Structure of the Succinyl-CoA Synthetase of *Escherichia coli*†

David Buck, Margaret E. Spencer, and John R. Guest*

Department of Microbiology, University of Sheffield, Sheffield S10 2TN, U.K.

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ABSTRACT: The primary structure of the succinyl-CoA synthetase of *Escherichia coli* has been deduced from the nucleotide sequence of a 2451-base-pair segment of DNA containing the corresponding *sucC* (β subunit) and *sucD* (α subunit) genes. The genes are located at one end of a gene cluster that encodes several citric acid cycle enzymes: $\overleftarrow{gltA\text{-}sdhCDAB}\text{-}\overrightarrow{sucABCD}$; *gltA*, citrate synthase; *sdh*, succinate dehydrogenase; *sucA* and *sucB*, the dehydrogenase (E1) and succinyltransferase (E2) components of the 2-oxoglutarate dehydrogenase complex. The *sucC* and *sucD* genes are separated from the *sucA* and *sucB* genes by a 273-base-pair segment containing four palindromic units, but they appear to be expressed from a *sucABCD* read-through transcript that extends from the *suc* promoter to a potential ρ-independent terminator at the distal end of *sucD*. The stop codon of the *sucC* gene overlaps the *sucD* initiation codon by a single nucleotide, indicating close translational coupling of the *sucC* and *sucD* genes. The *sucC* gene comprises 1161 base pairs (388 codons, excluding the stop codon), and it encodes a polypeptide of M_r 41 390 corresponding to the β subunit of succinyl-CoA synthetase. The *sucD* gene comprises 864 base pairs (288 codons, excluding the start and stop codons), and it encodes a product of M_r 29 644, corresponding to the α subunit of succinyl-CoA synthetase. The α subunit contains a 12-residue amino acid sequence that is identical with the histidine peptide previously isolated from the phosphoenzyme. This sequence forms part of one of the two potential nucleotide binding sites detected in the α subunit.

The succinyl-CoA synthetase (SCS)[1] of *Escherichia coli* (EC 6.2.1.5) catalyzes the following reaction via three steps that involve phosphoryl enzyme and enzyme-bound succinyl phosphate as intermediates:

$$\text{succinate} + \text{CoA} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{succinyl-CoA} + \text{ADP} + P_i$$

During aerobic metabolism it functions in the citric acid cycle coupling the hydrolysis of succinyl-CoA to the synthesis of ATP and thus represents an important site of substrate-level phosphorylation. It can also function in the other direction for anabolic purposes, and this may be particularly important for providing succinyl-CoA during anaerobic growth when the oxidative route from 2-oxoglutarate is severely repressed.

The *E. coli* enzyme has a molecular weight of 140 000 and comprises two types of subunit assembled as an $(\alpha\beta)_2$ tetramer

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* Author to whom all correspondence should be addressed.

[1] Abbreviations: CoA, coenzyme A; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; ODH, 2-oxoglutarate dehydrogenase; CS, citrate synthase; AK, adenylate kinase; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; IS, insertion sequence; REP, repetitive extragenic palindrome; kbp (kb in Figure 1), kilobase pair; kDa, kilodaltons. The standard single-letter code is used for amino acids.

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(Bridger, 1974; Vogel & Bridger, 1982). The α subunit (M_r 29 500) binds ATP and catalyzes phosphoryl transfer to one of its histidine residues, whereas the β subunit (M_r 38 500) contains the attachment sites for succinate and CoA. The complete active site is probably located in the region of α–β contact (Bridger, 1974; Pearson & Bridger, 1975; Collier & Nishimura, 1979). The enzyme exhibits two interesting properties: "substrate synergism", in which the enzyme is most active for the catalysis of its partial reactions only when all the substrate binding sites are occupied, and "catalytic cooperativity" between alternating active sites in the tetramer, whereby the interaction of substrates (particularly ATP) at one site is needed to promote catalysis at the other (Bridger et al., 1968; Bridger, 1984). The succinyl-CoA synthetases of eukaryotes and Gram-positive bacteria differ from the Gram-negative enzymes in containing single $\alpha\beta$ dimers (M_r ~70 000), and the enzymes from different sources exhibit a range of nucleotide specificities (Weitzman, 1981). The *E. coli* enzyme has recently been crystallized as a first step toward defining its three-dimensional structure (Wolodko et al., 1983).

No mutants of *E. coli* lacking SCS have been characterized, and of all the citric acid cycle genes only those encoding SCS and aconitase have still to be identified. However, evidence has accumulated that the SCS genes are distal genes in the same operon as the *suc* genes that encode the 2-oxoglutarate dehydrogenase (ODH) complex at 16.8 min in the *E. coli* linkage map (Bachman, 1983). First, it was observed that SCS expression is reduced in *sucA* amber mutants, but whether this represents transcriptional polarity or is simply a consequence of the deficiency in ODH complex was not established (Creaghan & Guest, 1972). Then, following the isolation of a segment of DNA containing the citrate synthase (*gltA*), succinate dehydrogenase (*sdh*), and *sucA* and *sucB* genes, (Spencer & Guest, 1982), the *sucAB* region was sequenced (Darlison et al., 1984; Spencer et al., 1984), and the start of another gene was detected downstream of *sucB*. The intergenic region contained no obvious promoter sequence, but four tandemly repeated palindromic units (REP) of the type formerly thought to function as intercistronic transcriptional attenuators (Higgins et al., 1982) were identified. Further evidence that the *suc* operon may contain one or more additional genes has come from transcription analysis in which a read-through transcript extending from *sucB* into a distal gene (*sucC*) and an independent *sucAB* transcript terminating at *sucB* were found (Spencer & Guest, 1985). These observations have prompted a detailed investigation of the region immediately downstream of the *sucB* gene, and this paper reports the complete nucleotide sequence of a 2451-base-pair segment of DNA containing the structural genes for β subunit (*sucC*) and α subunit (*sucD*) of succinyl-CoA synthetase.

Materials and Methods

Materials. Restriction endonucleases, T_4-DNA ligase, and T_4-DNA polymerase were obtained from Bethesda Research Laboratories (Cambridge, U.K.) and nuclease S1, DNA polymerase (Klenow fragment), and dideoxynucleotide triphosphates from Boehringer Corp. Ltd. (Lewes, U.K.). All enzymes were used according to the manufacturers' instructions. The M13mp8-11, 18, and 19 RF DNAs were purchased from P-L Biochemicals (Northampton, U.K.) and the 17-mer primer was from Celltech (Slough, U.K.). Deoxyadenosine 5′-[α-^{35}S]thiotriphosphate and U-^{14}C-labeled protein hydrolysate were supplied by Amersham International.

Sources of DNA. The relevant segment of DNA was originally cloned in λG118, a λ*gltAsdhsucAB*-transducing phage (Spencer & Guest, 1982). Several fragments were then subcloned into appropriate sites in the *tet* gene of plasmid pBR322 to provide derivatives that could be used as sources of DNA for detailed restriction site mapping and for the sequence analysis. The relevant fragments and plasmids are shown in Figure 1: pGS64, 5.4-kbp *Bam*HI–*Sal*I (B_3–Sa_2); pGS128, 7.0-kbp *Xho*I–*Sal*I (X-Sa_2); pGS130, 2.3-kbp *Sal*I–*Bgl*II (Sa_2–Bg_5); pGS131, 4.5-kbp *Bgl*II–*Bgl*II (Bg_4–Bg_5). The *tet* promoter has the same polarity as the *suc* genes in every case except pGS130. The methods used for preparing phage and plasmid DNA have been reported previously (Stephens et al., 1983). Specific fragments were isolated from agarose gels by using DEAE-cellulose paper (Dretzen et al., 1981).

Cloning in M13. The nucleotide sequence was derived from M13 clones obtained by a combination of "shotgun" and "forced" cloning of DNA fragments into appropriate cloning sites of the M13mp8-19 vectors (Messing & Vierra, 1982; Norrander et al., 1983). The products of digesting the 2.5-kbp *Nru*I fragment of pGS131 (N_5–N_6 in Figure 1) with *Msp*I or *Sau*3A were ligated directly into the *Acc*I or *Bam*HI sites of M13mp8. Ultrasonic fragments of the Ba_3–Sa_2 segment of pGS64 were generated during the sequencing of the *sucA* and *sucB* genes; they were blunt-ended with T_4-DNA polymerase and cloned into *Sma*I-cut and phosphatase-treated M13mp8 (Darlison et al., 1984; Deininger, 1983). The specific fragments, their sources, and the vectors into which they were cloned were as follows: the *Bgl*II–*Sal*I fragment (Bg_4–Sa_2) of pGS64 in M13mp8 and M13mp9; the *Cla*I fragment (C_{pBR322}–C_2) of pGS64 in M13mp8 (C_1 is a *Cla*I site that is not cleaved in Dam-methylated DNA); the *Sal*I–*Bcl*I fragment (Sa_2–Bc) of pGS130 in M13mp18; the *Cla*I fragment (C_2–C_3) of pGS131 in M13mp10 and M13mp11. Several strategies were used to isolate specific clones in the final stages in order to obtain sequence from both DNA strands. Thus, small *Sau*3A–*Sal*I and *Taq*I–*Msp*I fragments were cloned into M13mp19 and M13mp18 from the corresponding digests of the Sa_2–Bc fragment of pGS130. Likewise, the clones marked a–c in Figure 1, containing *Xho*II–*Taq*I, *Sau*3A–*Cla*I, and *Sau*3A–*Sal*I fragments in M13mp19, were isolated by hybridizing single-stranded DNA from appropriate M13 phages containing complementary inserts, treating with nuclease S1, and then cloning from digests of the residual double-stranded fragment (Miles & Guest, 1985). Also for the same purpose, a few of the shotgun clones were "turned round" by the procedure of Winter & Fields (1980) using M13mp18 and 19. One such phage containing an *Msp*I fragment generated a blue plaque because the cloning resulted in an in-phase fusion between the β subunit of SCS and the α peptide of β-galactosidase. Recombinant phages were identified as colorless plaques on indicator plates containing 5-bromo-4-chloro-3-indolyl β-D-galactoside, after transfecting *E. coli* strain JM101 according to published procedures (Sanger et al., 1980).

Nucleotide Sequence Analysis. Single-stranded M13 DNA templates were prepared and sequenced by the dideoxy chain-termination method using a synthetic 17-mer primer (Sanger et al., 1977). All clones were screened initially by A tracking, and the sequence data were compiled and analyzed by using the computer programs of Staden (1977, 1979, 1980) and Staden & McLachlan (1982).

Enzymology. Succinyl-CoA synthetase specific activities [μmol min^{-1} (mg of protein)$^{-1}$] were assayed spectrophotometrically at 230 nm according to Bridger et al. (1969) and Creaghan & Guest (1972) using ultrasonic extracts of exponential cultures of *E. coli* strain ED8641 (*hsdR supE recA56 met trp*) transformed with pBR322 or its derivatives and grown

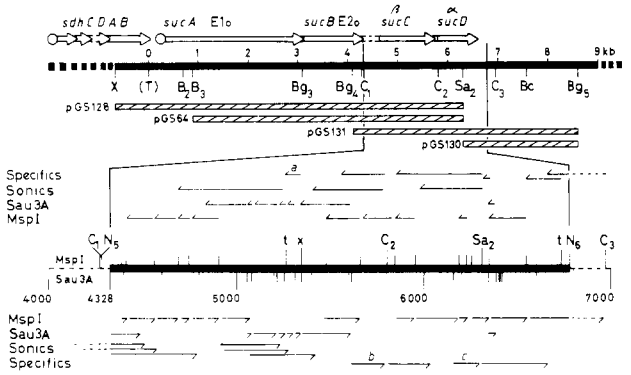


FIGURE 1: Locations of the genes encoding the α and β subunits of succinyl-CoA synthetase and summary of the DNA sequence data obtained from M13 clones. The upper lines show the positions and polarities of the *sdh* and *suc* genes aligned with the restriction map: left to right corresponds to clockwise orientation of the 16.8-min region of the *E. coli* linkage map. The restriction sites for *Bam*HI (B), *Bcl*I (Bc), *Bgl*II (Bg), *Cla*I (C), *Nru*I (N), *Sal*I (S), and *Xho*I (X) are numbered according to Spencer & Guest (1982) but modified to account for the *Bam*HI and *Bgl*II sites which have since proved to be paired (B_2,B_3 and Bg_1,Bg_2). The segments of DNA cloned into the *tet* genes of pBR322 are indicated by hatched bars. The lower lines summarize the polarities and extents of the sequence data obtained from M13 clones containing parts of the *sucCD* region. The segments marked a–c were isolated by hybridizing complementary clones (see Materials and Methods), and some additional restriction sites used in isolating specific clones are indicated: *Taq*I (t) and *Xho*II (x). The nucleotide positions are numbered in base pairs from the first base of a *Taq*I site (T) at the end of the *sdhB* gene (Darlison et al., 1984).

in glucose-free L broth with ampicillin (50 μg/mL).

Maxicell Procedure. Polypeptides expressed from plasmid-borne genes were labeled with ^{14}C L-amino acids by the maxicell procedure (Sancar et al., 1979; Shaw & Guest, 1982) using cultures of *E. coli* strain AB2480 (*uvrA6 recA13 pro-2 thi-1 lac rpsL*) that had been transformed with the relevant plasmids. The radioactive polypeptides were analyzed fluorographically following sodium dodecyl sulfate–polyacrylamide gel electrophoresis and treatment with Enlightening (New England Nuclear, Southampton, U.K.).

Results and Discussion

Location of the Succinyl-CoA Synthetase Genes. A map illustrating the organization of several citric acid cycle genes in the segment of bacterial DNA cloned in λG118 (λ*gltA-sucB*) is shown at the top of Figure 1. Also shown are several of the subfragments that have been cloned into the *tet* gene of pBR322, in order to identify the genes located immediately downstream of those encoding the specific E1 and E2 components of the 2-oxoglutarate dehydrogenase complex (*sucA* and *sucB*). Transcription–translation studies with maxicells showed that pGS131 expresses three bacterial polypeptides (M_r 41, 31, and 30 kDa) of which only the largest is expressed from pGS128 and pGS64 and the smallest from pGS130 (D. Buck, unpublished results). This indicated that there are two genes, now designated *sucC* and *sucD*, encoding polypeptides of M_r 41 and 31 kDa, respectively, in the 2.5-kbp segment of DNA adjacent to the *sucAB* region (Figure 1). The sizes of the gene products are close to those of the β and α subunits of SCS, and this tentative identification was confirmed when it was found that the specific activity of SCS is increased 8-fold from 0.2 μmol min^{-1} (mg of protein)$^{-1}$ in strains harboring pBR322, pGS128 or pGS130 to 1.6 μmol min^{-1} (mg of protein)$^{-1}$ with pGS131.

Sequencing Strategy. The strategy adopted for extending the sequence of the *suc* region involved a combination of shotgun and forced cloning of a variety of fragments from the Bg_4–Bg_5 region, particularly the 2.5-kbp *Nru*I fragment (N_5–N_6) of pGS131 (Figure 1). The M13 clones used for sequencing the 2451-base-pair segment of DNA containing the *sucC* and *sucD* genes are summarized in Figure 1, and the complete and unambiguous nucleotide sequence is presented in Figure 2. The sequence is fully overlapped, all of it was obtained from several independent clones, and 98.9% (100% of the coding region) was derived from both strands of DNA. The sequence runs from an arbitrary *Sau*3A site (position 4328) in the *sucB* gene to a *Nru*I site (N_6; position 6773) just downstream of *sucD*, and it extends the sequence of the *suc* region by a total of 2158 base pairs.

Identification of Coding Regions. The coding regions were identified by using the computer program FRAMESCAN (Staden & McLachlan, 1982), which predicts the location of structural genes by a statistical analysis of codon usage in all six reading frames. Two open reading frames having the same polarity as the *suc* operon were found at positions 4631–5794 and 5797–6663 (Figure 2). Both start with AUG codons that are located in regions corresponding to translational initiation sites when analyzed by the perceptron algorithm of Stormo et al. (1982). The open reading frames are terminated by ocher (UAA) codons, and both exhibit consistently high scores with respect to codon preference when a variety of *E. coli* genes are used as standards. Furthermore, their relative positions and sizes of gene product (M_r 41 390 and 29 644) agree with those predicted from the "maxicell" studies. The evidence therefore suggests that they correspond to the *sucC* and *sucD* genes encoding the respective β and α subunits of succinyl-CoA synthetase. The complementary strand contained several stretches of open reading frame, but none had any of the properties of coding regions.

Features of the Nucleotide Sequence. Previous results have indicated that the *suc* genes are expressed from two transcripts that are initiated at position 207 by a *suc* promoter (Spencer & Guest, 1985). One is a 4382-base *sucAB* transcript terminating at position 4588 (Figures 1 and 2), whereas the other is longer and extends into the *sucCD* region. No potential promoters were detected in the 273-base-pair *sucB–sucC* intergenic region or the region upstream of *sucD* when searched with a perceptron analysis based on the sequence of known promoters (Hawley & McClure, 1983; Spencer & Guest, 1985). The sequence was also examined for regions of hyphenated dyad symmetry, and those capable of forming stable stem-and-loop structures in the transcript are shown in Figure 2. Apart from the four components of the palindromic unit (aa′, bb′, cc′, and dd′), the most significant is just downstream of the *sucD* gene: ee′ at position 6742–6759 (ΔG = −17.2 kcal mol^{-1}; Tinoco et al., 1973). This has a GC-rich stem followed by a run of six T's, strongly resembling a ρ-independent terminator sequence (Rosenberg & Court, 1979), and there is evidence from preliminary transcript mapping that a *suc* mRNA terminates here. These observations suggest that the *sucC* and *sucD* genes are cotranscribed and, consistent with earlier failures to detect an independent *sucCD* transcript, it would appear that they are normally transcribed as part of a long *sucABCD* read-through transcript that extends for almost 6560 bases. The amplification of SCS with pGS131 can be attributed to transcription of the *sucCD* genes from the flanking *tet* promoter. The 22-base-pair consensus sequence of Chapon & Kolb (1983) was used to search for sites of cyclic AMP receptor protein (CRP) interaction, generally associated with the promoters of genes that are subject to catabolite repression, but none was found.

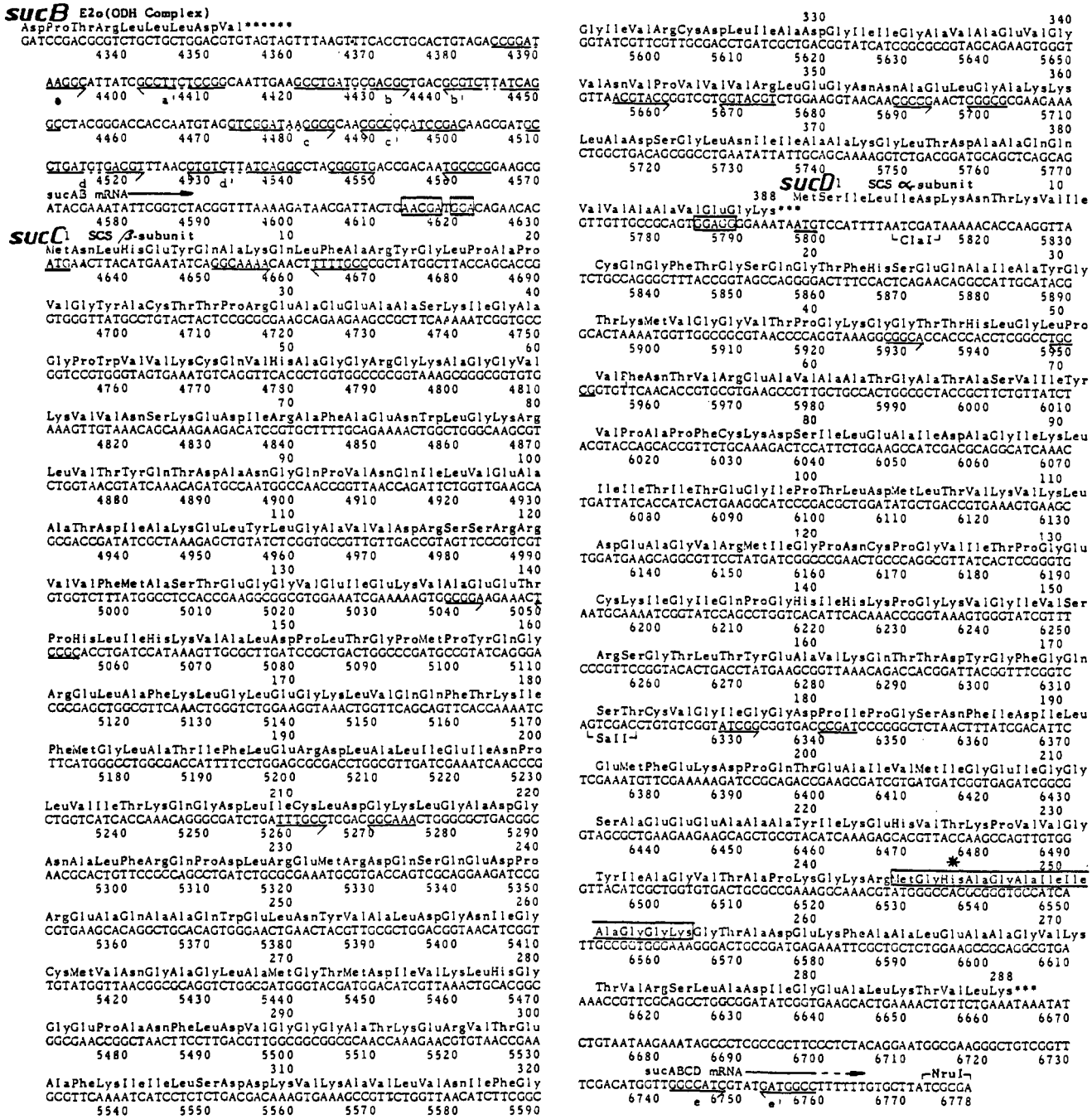


FIGURE 2: Nucleotide sequences of the *sucC* and *sucD* genes and the primary structures of the β and α subunits of the *E. coli* succinyl-CoA synthetase. The nucleotide sequence (2451 base pairs) of the noncoding (sense) strand is numbered from a convenient *Taq*I site upstream of the *sucA* gene (see Figure 1) and extends the previous sequence of the *suc* operon by 2158 base pairs. The amino acid sequences of the β and α subunits of SCS are numbered from the initiating methionine (β, 388 residues) or from the adjacent residue (α, 288 residues). Potential ribosome binding sites (boxed), translational initiation sites (underlined), and relevant stop codons (asterisk) are indicated. Significant regions of hyphenated dyad symmetry are marked by converging arrows and include four members of the intergenic palindromic unit (aa′, bb′, cc′, and dd′) and a potential ρ-independent terminator (ee′). Other features include arrows showing the 3′ limits of mRNA transcripts, the histidine residue (asterisk) in the phosphopeptide (boxed) of the α subunit, and relevant restriction sites.

The *sucC* structural gene is preceded by two potential ribosome binding sites having five and three consecutive bases that are complementary to the 3′-terminal sequence of 16S ribosomal RNA (Figure 2). The latter, GATGGACAGAA (positions 4617–4627), is better placed with respect to the initiation codon. The initiation codon of the *sucD* gene overlaps the *sucC* stop codon by a single base, and there is a well-placed ribosome binding site AGTGGAGGGGA (positions 5782–5792) at the distal end of the *sucC* coding region. The close proximity of the *sucC* and *sucD* genes probably ensures that the β and α subunits are produced in equimolar proportions. Other examples of this type of translational coupling are to be found in the *trp* operon (*trpE-D*, *trpB-A*; Yanofsky et al., 1981) and the *frd* operon (*frdA-B*; Cole et al., 1982). This contrasts with the large 273-base-pair interval between *sucAB–CD* segments of the operon and may provide a mechanism for differential synthesis of the ODH complex and SCS from the *sucABCD* transcript, in addition to that mediated by the synthesis of an independent *sucAB* transcript.

The codon usage for *sucC* and *sucD* is nonrandom (Table I) and typical of *E. coli* genes that are strongly or moderately expressed (Grosjean & Fiers, 1982). In the eight pairs of codons (boxed in Table I) where the preference for U or C in the third position is particularly correlated with the level

Table I: Codon Usage in the *sucC* and *sucD* Genes[a]

	sucC	D		sucC	D		sucC	D		sucC	D	
UUU F	3	2	UCU S	1	2	UAU Y	6	1	UGU C	3	1	
UUC F	8	6	UCC S	2	4	UAC Y	1	5	UGC C	2	4	
UUA L	2	1	UCA S	1	1	UAA -	1	1	UGA -	0	0	
UUG L	1	0	UCG S	1	1	UAG -	0	0	UGG W	3	0	
CUU L	3	0	CCU P	1	1	CAU H	2	0	CGU R	10	4	
CUC L	4	2	CCC P	0	0	CAC H	3	6	CGC R	7	1	
→ CUA L	0	0	CCA P	1	4	CAA Q	3	0	CGA R	0	0	←
CUG L	28	11	CCG P	12	10	CAG Q	14	7	CGG R	0	0	←
AUU I	5	7	ACU T	4	8	AAU N	2	0	AGU S	1	0	
AUC I	18	23	ACC T	7	15	AAC N	14	4	AGC S	2	3	
→ AUA I	0	0	ACA T	1	1	AAA K	22	19	AGA R	0	0	←
AUG M	8	6	ACG T	3	2	AAG K	2	3	AGG R	0	0	←
GUU V	18	12	GCU A	10	8	GAU D	7	7	GGU G	18	19	
GUC V	3	1	GCC A	10	7	GAC D	14	4	GGC G	23	19	
GUA V	7	2	GCA A	15	7	GAA E	24	15	GGA G	1	0	←
GUG V	10	9	GCG A	12	7	GAG E	4	3	GGG G	1	3	←

[a] The codon pairs whose use varies particularly between strongly and weakly expressed genes are boxed, and rarely used (modulating) codons are arrowed.

Table II: Amino Acid Composition of Succinyl-CoA Synthetase[a]

	β subunit		α subunit		$(\alpha\beta)_2$ enzyme	
amino acid	gene	protein	gene	protein	gene	protein
Asp	21	34.2	11	17.3	64	104.7
Asn	16		4		40	
Thr	15	15.6	26	25.2	82	77.9
Ser	8	9.5	11	12.5	38	39.1
Glu	28	44.1	18	28.7	92	135.2
Gln	17		7		48	
Pro	14	13.0	15	15.7	58	53.8
Gly	43	42.7	41	42.9	168	159.3
Ala	47	43.1	29	30.0	152	143.5
Val	38	37.0	24	26.4	124	115.6
Met	8	7.6	6	6.6	28	26.7
Ile	23	21.3	30	28.8	106	94.0
Leu	38	36.1	14	16.4	104	103.2
Tyr	7	6.7	6	6.3	26	22.1
Phe	11	10.5	8	8.4	38	38.4
Lys	24	22.6	22	21.4	92	88.0
His	5	4.0	6	4.8	22	19.1
Arg	17	15.3	5	6.0	44	42.8
Cys	5		5		20	23.7
Trp	3		0		6	8.2
total	388		288		1352	
M_r	41390	38500	29644	29500	142068	136000
polarity (%)	39		38			

[a] The amino acid compositions of SCS and its subunits derived from the nucleotide sequence are compared with those reported for the proteins by Bridger (1974).

of gene expression, 58.8% (*sucC*) and 64.4% (*sucD*) are of the strongly expressed class and closely correspond to the preferences found in *sucA* (62.3%) and *sucB* (68.1%). Furthermore, the *sucC* and *sucD* genes use very few (two *sucC*; three *sucD*) of the rare, potentially modulatory, codons which correspond to the minor tRNA species (arrowed in Table I).

The *sucC* gene contains a chi site (positions 4781–4788; Figure 2). This enhances generalized recombination and the production of λ*redgam* phages, so its presence in the *sucC* gene could explain why a high proportion of the λ*gltAsdh* phages generated by excision of λ*gltA* prophages had also incorporated the *suc* genes (Spencer & Guest, 1982).

Structures and Compositions of the α and β Subunits of SCS. The primary structures of the α and β subunits are shown above the nucleotide sequence in Figure 2. The amino-terminal residues, serine (α) and methionine (β), agree with those identified previously (Bridger, 1974), assuming that the initiating formylmethionine is removed posttranslationally from the α but not the β subunit. The molecular weights and amino acid compositions (Table II) are in excellent agreement with

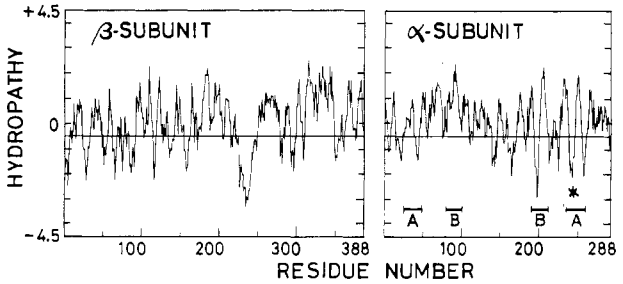


FIGURE 3: Hydropathy profiles of the β and α subunits of SCS. Consecutive hydropathy averages are plotted for a seven-residue window advancing from amino- to carboxy-terminus. Relative hydrophobicity and hydrophilicity are recorded in the range +4.5 to −4.5 (Kyte & Doolittle, 1982), the horizontal line being the average for most sequenced proteins. The position of the phosphohistidine residue (asterisk) and potential nucleotide binding sites (A and B sequences) are indicated.

those obtained with the native enzyme and the isolated subunits (Bridger, 1974). The α subunit is particularly rich in glycine and deficient in asparagine, glutamine, and serine. The β subunit also has a high glycine content and relatively little serine. The cysteine content is consistent with estimates of 16–18 free sulfhydryl groups per $\alpha_2\beta_2$ tetramer (Nishimura et al., 1973), if no more than four or two are involved in disulfide bridges. An amino acid sequence corresponding to the tryptic phosphopeptide of the α subunit (Bridger, 1974) is found at residues 244–255 (Figure 2) and confirms the identification of the *sucD* gene product.

Hydropathy profiles, which are of value in predicting secondary structural features, are shown in Figure 3. There are segments of ~150 residues at the carboxy-terminal (β) or amino-terminal (α) ends of each subunit which appear particularly hydrophobic. The β subunit also has a fairly extensive hydrophilic segment (residues 225–245), and it may be significant that the phosphohistidine residue of the α subunit is in a hydrophilic region flanked by two hydrophobic segments. The secondary structures predicted by the method of Garnier et al. (1978) are 38% α-helix and 43% β-sheet for the α subunit and 47% α-helix and 39% β-sheet for the β subunit.

Sequence Homologies and Structural Features. Sequence homologies between SCS and a variety of enzymes that interact with the same substrates were sought by visual inspection and using the computer program DIAGON, which finds highly significant regions of homology based on the MDM_{78} scoring matrix (Staden, 1982). These enzymes included three citrate synthases (Ner et al., 1983; Suissa et al., 1984; Bloxham et al., 1981; Remington et al., 1982), dihydrolipoamide acetyltransferase and succinyltransferase (Spencer et al., 1984), succinate dehydrogenase and fumarate reductase (Wood et al., 1984), adenylate kinase (AK), the α and β subunits of the *E. coli* ATP synthase, and a variety of other ATP- or GTP-utilizing enzymes. No extensive or sustained alignments indicative of an evolutionary relationship between either subunit of SCS and any of the other enzymes were detected.

In the case of the ATP binding site, comparisons can be made with AK, where the tertiary structure has been defined (Pai et al., 1977), and two sequences (A and B) that contribute to its AMP-binding pocket are conserved in the α and β subunits of ATP synthase and in other enzymes that utilize ATP (Walker et al., 1982; Finch & Emmerson, 1984). In SCS (α) there are two segments that contain the G/A-X_4-G-K-T/G consensus of the A sequences, and one of the segments includes the histidine residue (246) which is phosphorylated during catalysis (Table III). In AK, five hydrophobic residues (10–14) form a β-sheet, which makes contact with the adenine

Table III: Alignments of Homologous Sequences in the α Subunit of SCS and Other Nucleotide Binding Proteins[a]

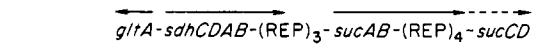
protein	residues	sequences	ref
		0 5 *10 15 20 25	
A. SCS α subunit	237-263	T A P K G K R M G H A G A I I A G G K G T A D E K F A	a
SCS α subunit	24-50	S E Q A I A Y G T K M V G G V T P G K G G T T H L G L	a
Adenylate kinase pig	3-29	E K L K K S K I I F V V G G P G S G K G T Q C E K I V	b
ATPase α subunit	158-183	P I G R G Q R E L I I - G D R G T G K T A L A I D A I	c
Rho protein	167-192	P I G R G Q R G L I V - A P P K A G K T M L L Q N I A	d
DNA helicase II	18-43	V A A P R S N L L V L - A G A G S G K T R V L V H R I	d
RecA protein	54-83	G G L P M G R I V E I Y G P E S S G K T T L T L Q V I	c
Myosin rabbit	166-192	L T D R E N Q S I L I T G E S G A G K T V N T K R V I	c
EF-G	4-30	T P I A R Y R N I G I S A H I D A G K T T T T E R I L	e
EF-Tu	6-32	E R T K P H V N V G T I G H V D H G K T T L T A A I T	e
B. SCS α subunit	191-213	L E M F E K D - P Q T E A I V M I G E I G G S A	a
SCS α subunit	80-102	S I L E A I D - A G I K L I I T I T E G I P T L	a
Adenylate kinase pig	102-124	G E E F E R K - I G Q P T L L L Y V D A G P E T	b
Phosphofructokinase Bacillus	85-107	G I E Q L K K - H G I Q G L V V I G G D G S Y Q	f
ATPase α subunit	265-287	M G E Y F R D - R G E D A L I I Y D D L S K Q A	c

[a] Conserved regions are boxed, and other potentially important residues are underlined. The references are the following: (a) this work; (b) Heil et al. (1974); (c) Walker et al. (1982); (d) Finch & Emmerson (1984); (e) Zengel et al. (1984); (f) Kolb et al. (1980); or references cited therein. The organism is *E. coli* unless specified.

ring, a glycine-rich region (15–22) makes a loop enclosing the α-phosphate, and the conserved lysine (21) forms a salt bridge with the α-phosphate (Table III). These features are preserved in most of the proposed A sequences (Table III). Secondary structure predictions (Chou & Fasman, 1974; Garnier et al., 1978) indicate that residues 32–35 in SCS form a β-sheet followed by a turn. A β-sheet is not predicted for residues 244–248, and it seems unlikely that these residues will form contacts with the adenine ring because the histidine residue (246) must be close to the γ-phosphate of ATP. Thus, it would appear that segment 24–50 in SCS more closely resembles the structure in AK. The B sequences are characterized by a K/R-X_{2-3}-G-X_3-(hydrophobic)$_4$ consensus followed by a negatively charged residue that is implicated in Mg^{2+} binding (Table III). In AK the hydrophobic residues form a β-sheet making contacts with the other face of the adenine ring, and the β-sheet is flanked by two α-helixes. Two sequences in the α subunit of SCS are predicted to have similar structures and could therefore form the other half of the ATP-binding sites (Table III). Because the SCS of *E. coli* will use GTP, albeit less effectively than ATP (Weitzman, 1981), the α subunit was compared with the GTP-utilizing elongation factors, EF-G and EF-Tu (Zengel et al., 1984). The latter contain homologous segments that closely resemble the A sequences, indicating that the underlying features of the adenine and guanine nucleotide binding sites are similar (Table III). It is also conceivable that the nucleotide moiety of CoA could interact with the α subunit across the α–β interface at a site resembling a nucleotide-binding site.

The CoA binding site of CS comprises a glycine-rich adenine-binding loop and various basic residues which stabilize the 3′-phosphate and 5′-diphosphate groups of CoA (Remington et al., 1982; Suissa et al., 1984). A consensus for the loop and one of the 5′-diphosphate contacts is V/L-X-G-X-G-X-X-V-X-R/K. Several glycine-rich segments of SCS come fairly close in the β subunit (residues 37–46, 55–64, 263–272, and 288–297) and in the α subunit (the A sequences). The enzyme is thought to contain two sulfhydryl groups per $\alpha\beta$ dimer that are important for interacting with CoA and ATP–Mg^{2+}–succinate (Nishimura et al., 1976). The former is in the β subunit close to a histidine residue (Collier & Nishimura, 1979). The corresponding residues could be cysteine-47 and histidine-50, and their proximity to two of the potential CoA binding sites may be significant. The succinate binding site of SDH is thought to comprise two basic residues separated by eight residues (Kotlyar & Vinogradov, 1984; Wood et al., 1984). There are many such sites in the β subunit, near the putative CoA binding sites and in the highly hydrophilic segment (residues 225–245; Figure 3).

Organization and Evolution of the Citric Acid Cycle Gene Cluster. The discovery and sequencing of the genes encoding SCS (*sucC* and *sucD*) adds two more genes not only to the *suc* operon but also to the main cluster of citric acid cycle genes. This now forms a continuous sequence of 13 061 base pairs containing nine genes that encode four enzymes or enzyme complexes. These genes are apparently organized in three transcriptional units:



The significance of the clustering and the presence of the REP sequences is uncertain. The *sdh* and *suc* genes mediate the conversion of 2-oxoglutarate to fumarate in three successive steps that are coordinately induced during aerobic respiration and repressed by anaerobiosis and glucose. When this route is repressed, succinate is generated by an anaerobically derepressed fumarate reductase, but it is not known whether sufficient succinyl-CoA is formed from 2-oxoglutarate or succinate by a very low level of expression of the *suc* operon or from succinate by the expression of the *sucCD* genes from a transcript that has escaped detection. The proximity and coexpression of the *sucAB* and *sucCD* genes is also interesting in view of the specific association between the mitochondrial ODH complex and SCS (Porpaczy et al., 1983), as it may facilitate the formation of an analogous complex in *E. coli*.

It is now clear that the IS-like REP sequences are not necessarily intercistronic, nor do they appear to have a unique function such as transcriptional attenuation (Gilson et al., 1984; Stern et al., 1984). The REP sequence in the *suc* operon is known to be transcribed in both short (*sucAB*) and long (*sucABCD*) transcripts (Spencer & Guest, 1985), and it could be involved in regulating the expression of the distal genes. In contrast the REP sequence separating *sdh* and *suc* contains a transcriptional terminator for the *sdh* operon, and no read-through transcript has been detected. These palindromes may have played a role in chromosome or operon evolution, in which case the *sucAB* genes could have been inserted into their present site by transposition from another source, so as to form a cluster of functionally related and coregulated genes. It is even possible that such an insertion resulted in the disruption of a preexisting *sdh–sucCD* operon. This is particularly relevant because it has been proposed that the citric acid cycle may have evolved by linking together two routes of primitive anaerobic metabolism, the oxidative synthesis of

2-oxoglutarate (glutamate) and the reductive formation of succinate (succinyl-CoA), by introducing the ODH step and reversing the reduction of fumarate (Gest, 1981). No evidence was found to support the interesting suggestion that SCS and CS may be evolutionarily related because of their analogous substrates (acyl-CoA), related functions, and the incidence of "large" and "small" enzymes (Weitzman, 1981).

Conclusion

The work described provides the first amino acid sequences of the succinyl-CoA synthetase α and β subunits from any source. It also places the corresponding genes (*sucC* and *sucD*) in the distal segment of an operon (*sucABCD*) that encodes the specific dehydrogenase (E1) and acyltransferase (E2) components of the 2-oxoglutarate dehydrogenase complex of *E. coli*. This operon forms part of a cluster of nine citric acid cycle genes, contributing subunits to four enzymes or enzyme complexes, in a completely sequenced 13 061-base-pair segment of the bacterial chromosome. The site of enzyme phosphorylation is clearly identified as part of an ATP binding site that is homologous to other adenine and guanine nucleotide binding sites. The amino acid sequences represent an important step in defining the structural basis of its interesting catalytic properties, subunit interactions, and association with the 2-oxoglutarate dehydrogenase complex. Further work will be aimed at manipulating the genes to maximize enzyme amplification and investigating all aspects of the structure–function relationships using site-directed mutagenesis. This should be particularly fruitful once the crystallographic analysis of the enzyme has been completed. The mechanisms controlling the expression of SCS under different physiological conditions and factors that could affect its coupling to the ODH complex will also be examined.

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Structural Mapping of Fc Receptor Bound Immunoglobulin E: Proximity to the Membrane Surface of the Antibody Combining Site and Another Site in the Fab Segments†

Barbara Baird* and David Holowka
Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853
Received December 20, 1984; Revised Manuscript Received April 9, 1985

ABSTRACT: Resonance energy-transfer methods have been used to investigate the structure of immunoglobulin E (IgE) bound to its high-affinity receptor on plasma membrane vesicles derived from rat basophilic leukemia cells. The structural mapping of receptor-bound IgE was initiated in an earlier study [Holowka, D., & Baird, B. (1983) *Biochemistry 22*, 3475], and it is based on measuring the minimal distance from IgE sites that are selectively labeled with donor probes to a plane of amphipathic acceptors at the membrane surface. This paper describes the use of monoclonal IgE specific for 5-(dimethylamino)naphthalene-1-sulfonyl (DNS) to place a donor probe, DNS-L-Lys, in the antibody combining sites. The distance from these sites to the membrane surface was determined to be greater than 100 Å with two different amphipathic acceptor probes. Another site in the Fab segments of monoclonal IgE (anti-dinitrophenyl) could be labeled selectively with *N*-[4-[7-(diethylamino)-4-methylcoumarin-3-yl]phenyl]maleimide (CPM) in the absence of reducing agents [CPM(−)], and the reaction could not be blocked by prereaction with *N*-ethylmaleimide. The pattern of CPM(−)-labeled proteolytic fragments and the lack of fluorescence quenching by (trinitrophenyl)lysine in the antibody combining sites suggested the CPM(−)-labeled site to be in the $C_\epsilon1$ domain of IgE. The distance between this site on receptor-bound IgE and the membrane surface was determined to be 75-87 Å with two different amphipathic acceptors. When IgE was labeled with CPM at the inter heavy chain disulfides in $C_\epsilon2$ in the presence of reducing agents [CPM(+)] and employed in further energy-transfer experiments, we observed an apparent interaction between this probe and one particular amphipathic acceptor but only when the IgE was bound to receptor. This suggests a conformational change in IgE accompanies receptor binding. The results from the experiments reported here support our previous findings and provide additional information about the structural orientation of receptor-bound IgE.

Immunoglobulin E (IgE)[1] binds very tightly to its receptor on mast cells and basophils and mediates the triggering of cellular degranulation by multivalent antigen. As shown schematically in Figure 1, IgE is similar to other classes of antibodies and is composed of two heavy (ϵ) and two light polypeptide chains that are connected by disulfide bonds as well as by nonconvalent interactions. Amino acid sequencing of human ϵ (Bennich & von Bahr-Lindstrom, 1974) and DNA sequencing of the rodent ϵ gene (Ishida et al., 1982; Liu et al., 1982) have shown IgE to have a domain structure that is quite similar to immunoglobulin G except that IgE has an extra domain, $C_\epsilon2$, in the region corresponding to the hinge of IgG.

Some information has been obtained regarding the manner in which IgE associates with its receptor. The binding region apparently is contained within the $(C_\epsilon2\text{-}C_\epsilon3\text{-}C_\epsilon4)_2$ domains (Ishizaka et al., 1970; Dorrington & Bennich, 1978), and more specific localization of this region has been suggested by proteolytic digestion studies with rodent IgE (Perez-Montfort & Metzger, 1982). These studies revealed a site of trypsin cleavage between the $C_\epsilon2$ and $C_\epsilon3$ domains on free rat IgE that

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[1] Abbreviations: IgE, immunoglobulin E; RBL, rat basophilic leukemia; HAF, 5-(hexadecanoylamino)fluorescein; ORB, octadecylrhodamine B; HAE, 5-(hexadecanoylamino)eosin; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; DNP, dinitrophenyl; TNP, trinitrophenyl; DNS, 5-(dimethylamino)naphthalene-1-sulfonyl; DNP-Lys, TNP-Lys, and DNS-Lys, L-lysine modified as indicated at the ϵ-amino group; IAEDANS, 5-[[[(iodoacetyl)amino]ethyl]amino]naphthalene-1-sulfonic acid; CPM, *N*-[4-[7-(diethylamino)-4-methylcoumarin-3-yl]phenyl]maleimide; CPM(+)IgE, IgE that has been first reduced with dithiothreitol and then alkylated with CPM; CPM(−)IgE, IgE that has been reacted with CPM without prior reduction; $NaDodSO_4$, sodium dodecyl sulfate.

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