

Short sequence-paper

The *coxD* gene for heme O synthase in *Synechocystis*¹

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Received 5 September 1995; revised 7 November 1995; accepted 15 November 1995

Abstract

The cyanobacterial *coxD* gene for heme O synthase was cloned from *Synechocystis* sp. PCC 6803 and its nucleotide sequence was determined. The deduced amino-acid sequence of the gene was homologous to the amino-acid sequences of bacterial heme O synthases. In contrast to the genes for heme O synthases in other prokaryotes, which are clustered together with genes for the structural subunit(s) of cytochrome oxidase, the *coxD* gene is not linked to such genes on the chromosome of *Synechocystis*.

Keywords: Cytochrome *c* oxidase; Heme O synthase; *CoxD* gene; (*Synechocystis* sp. PCC 6803)

In seven species of bacteria ([1–3] and references therein) the gene for heme O synthase, which catalyzes the conversion of reduced heme B to heme O [4,5], is found in a cluster with the gene for heme O oxygenase, which converts heme O to heme A [6], and with genes for the structural subunit(s) of *bo*-type quinol oxidase or *aa*₃-type cytochrome oxidase. However, in cyanobacteria such as *Synechococcus vulcanus* [7] and *Synechocystis* sp. PCC 6803 [8], the gene for heme O oxygenase is found in a cluster with the genes for the structural subunits of *aa*₃-type cytochrome oxidase, but the genes for heme O synthase are not found in regions adjacent to this cluster.

We report here the cloning of a gene from *Synechocystis* sp. PCC 6803 that is homologous to genes for heme O synthases in other bacteria. This gene, designated *coxD*, was found 9 kb downstream of the *desA* gene [9]. Cloning and sequencing were performed as described previously

[10]. Homology scores were calculated using the Dayhoff MDM-78 matrix [11] and the PCGene Program Package (IntelliGenetics, Geneva, Switzerland). The nucleotide and the deduced amino-acid sequences of the *coxD* gene are shown in Fig. 1. The gene encodes a protein of 316 amino-acid residues with a calculated molecular weight of 34 804. Southern blot analysis of the genomic DNA demonstrated that the gene is present as a single copy on the chromosome of *Synechocystis* sp. PCC 6803.

The alignment of the deduced amino-acid sequence of the *coxD* gene with the amino-acid sequences of heme O synthases from other organisms is shown in Fig. 2. Site-directed mutagenesis of 40 amino-acid residues in the heme O synthase of *Escherichia coli* (*cyoE*), reported by Saiki

Abbreviations: *CoxD*, the gene in *Synechocystis* sp. PCC 6803 that is homologous to *cyoE*; *CoxD*, the protein encoded by the *coxD* gene; *cyoE*, the gene in *Escherichia coli* for heme O synthase; *CyoE*, the protein encoded by the *cyoE* gene.

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¹ The nucleotide sequence data of the *coxD* gene have been deposited with the DDBJ/EMBL/GenBank databases under the accession number D13960.

Fig. 2. Alignment of the amino-acid sequence deduced from the *coxD* gene with the amino-acid sequences of other heme O synthases. Asterisks indicate identity between *CoxD* and other heme O synthases. The residues in *CyoE* that are essential for the function of the enzyme are indicated by # (invariant in *CyoE* and *CoxD*) or \$ (shifted or replaced by a homologous residue) (Saiki et al., 1993). Abbreviations: *CoxD*, heme O synthase of *Synechocystis* sp. PCC 6803 (this work); ORF1-*R.s.*, heme O synthase of *Rhodobacter sphaeroides* (pir A45164); ORF1-*P.d.*, heme O synthase of *Paracoccus denitrificans* (pir S03804); COX10-*S.c.*, heme O synthase of *Saccharomyces cerevisiae* (pir A37812); Cox10-*H.s.*, human heme O synthase (genpept HSU09466_1); CtaB-*B.s.*, heme O synthase of *Bacillus subtilis* (pir A14395); CtaB-*B.f.*, heme O synthase of *Bacillus firmus* OF4 (pir B45335); *CyoE*, heme O synthase of *Escherichia coli* (pir E42226).

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GGAATACTCGCTTAAAATTCAGGAAAAATTTTGTATCTCCCCCTGCTTACCCTTTCTTT 60
TGATTCTCTATGGTTACTTCGACAAAAATCCATCGCCAACACGATTCCATGGGGCGGTG 120
      M V T S T K I H R Q H D S M G A V 17
TGCAAAAGTTATTATCAGTTAACCACGAGAAATATCCCCCTCTTGCTGATTACCACC 180
C K S Y Y Q L T K P R I I P L L L I T T 37
GCTGCTCCATGTGGATTGCCTCGGAAGGTCGGGTGGATTACCCAAATGCTGATCACT 240
A A S M W I A S E G R V D L P K L L I T 57
CTGCTGGGAGAACCCCTTCAGCCGCTTCAGCCCCAACCCCTGAAGTGTATTACGACCAA 300
L L G G T L A A A S A Q T L N C I Y D Q 77
GACATTGACTATGAAATGCTCCGACACGAGGCCGACCCATCCAGCCGAAAAGTGCAA 360
D I D Y E M L R T R A R P I P A G K V Q 97
CCCCGCCATGCGTTGATTTTCGCCCTAGCCTTAGGGGTACTTTTCGTTGCTCTTTTGGCC 420
P R H A L I F A L A L G V L S F A L L A 117
ACCTTTGTCAATGTACTAAGCGGTTCCTAGCATTATCCGGCATTGTCTTTTACATGTTG 480
T F V N V L S G C L A L S G I V F Y M L 137
GTCTATACCCATTGGCTGAAACGACACACGCCCCAAACATTGTCTCGGTGGGGCTGCG 540
V Y T H W L K R H T A Q N I V I G G A A 157
GGTTCCATTCCCCCCTTGGTGGGCTGGGCGCTGTTACGGGGGATTTAAGCTGGACTCCC 600
G S I P P L V G W A A V T G D L S W T P 177
TGGGTACTGTTTGCCTAATTTTCTTGGACTCCCCCCTTTTGGGCCCTGGCTTTG 660
W V L F A L I F L W T P P H F W A L A L 197
ATGATCAAGGATGATTATGCCCAGGTGAATGTCCCATGTTGCCGGTAATTGCGGGGGAG 720
M I K D D Y A Q V N V P M L P V I A G E 217
GAAAAACCGTTAGCCAAATCTGGTACTACTACTGTTGGTGGTGCCTTTTCAAGTTGCTG 780
E K T V S Q I W Y Y S L L V V P F S L L 237
CTAGTTTATCCTCTGCACCAAGTGGGCATACTTTATCTGGCGATCGCCATTATTTTGGGG 840
L V Y P L H Q L G I L Y L A I A I I L G 257
GGACAATTTCTGGTCAAAGCTTGGCAACTCAAACAGGCTCCCGCGATCGGGATTGGCT 900
G Q F L V K A W Q L K Q A P G D R D L A 277
AGGGGATTTGTTCAAGTTCTCCATTTTACCTAATGTTGCTGCTGCGTGGCCATGGTGATT 960
R G L P K F S I F Y L M L C L A M V I 297
GACAGTTTGGCCGTCACCCATCAGTTGGTGCCCAAATGGGAACCTTACTGTTGGGTTAA 1020
D S L P V T H Q L V A Q M G T L L L G * 316
AGTTGAACCATGGCCAGGTTTGGTTTTCTCAAAGCTCCACTGACTTATTGCCAATTC 1074

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Fig. 1. The nucleotide and the deduced amino-acid sequences of the *coxD* gene of *Synechocystis* sp. PCC 6803.

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CoxD      MVTSTKIHRQHDSMGA-----VCKSYVQLTK---PR--IIPLL-LI-TTAASMWIASEGRVDPKLLITLGGTLAAASAQTLNCIYDQDIDYEMLRTRARPAPAGKV
Cox10-H.s. VIEDSIDVQKETKEEKRWKEMKQVYDLPGLAQLSK---IKLTALVSVSTTAAGFALAP---GPFDPWPCFLITSVGTGLASCAANSINQFEVFPDSNMNRKTNRPVLRGQI
Cox10-S.c. VDPMVRKSKRPSHAISEGLNMKTLKKVIMPYLQLTK---PRLTILVMSLSAICSYALSPYPAS---VN--ELLCLTVGTTLCGSGANAINMGREPEFDQMVRTQARFVVRGVD
ORF1-R.s.      MSL-VVFTALVGLLVAPVTVMHLDITMIFALLGAALVMAGGCTLNNYIDRDIHLMERTKERPTVTGRF
ORF1-P.d.      MSL-VVFTAFVGLWIAQPQVNPVFVAFCAVLFIAGGGAS-GALNMWYDADIDAVMRRTAGRPVPSGRV
CtaB-B.s.      MANS---RILNDTAIDGQIEETAWKDFLSLIKIGIVNSNLITFTGMSVALHISGLSFL-GNIN-TVLL-TLIGSSLIAGSCAINNWDYDRDIHLMERTKVRPTVTGKI
CtaB-B.f.      MNKSNTAIDPTNIEAGPDSSVADVQKSWKDYLVLAQKQIVTSNLIITFAGIYLAIVYTGTVFT--HPIALTGILFIAGGAS-GALNMWSHEDIDRVKRTNRNPVPSGTV
CyoE          MMFKQYLQVTK---PG--IIFGN-LI-SVIGFLLASKGSDYPLFIYTLVGVSLVVASGCVFNNYIDRDIKRMERTKNRVLVKGLI
              # # # # #
QPRHALIFALALGVLSFALLATFVNVLGCLALSIGVIFMLVYTHWLKRHTAQNIIVIGGAAGSIPLVGVAAVTDGLSWTPVLFALIFLWTPPHFWALALMIKDDYQVNVPMPLVPIAGEEKTVSQ
SPLLAVSFATCCAVPGVAITLGVNPLTGALGLNFIPLYTCCYTP-LKRISIANWVGAVVGAIPVPMGWTATGSLDAGAFLLGGILYSWQFPFNALSWSGLREDYSRDGYCMMSVTHPGLCRRVA
TPTQAFEPAAIGTLGVSILYFGVNPVAILGASNIALYGYWYTS-MKRKHIINTWLGALVGMVPELMGWAAASPLSHPGSWCLAGLLFAWQFPFHNTLSHNIRNEYKNAGVYM--TAWNPLLNAR
SAKHVLVGLAQAALGIIIFLAL-TTPTAIVIGLIGLFIYVVLVTMWRKRTITLNTIIVGSESGAVPLIGMAAIDGGLHYAWLLFFINFLWQPPHFLALAMKRVVEYRAAGIPMLPVVAGFEMTKRQ
TSQEPLAVGIALSGLSVMMLGAGGNWFAAGFLAFTIFFYAVVYTIWLKRTSPQNIIVIGGAAGAFPPMIGWALPTGGIGIESLLMFALIFFWTTPPHFWALALFMKDDYSKAGVPMILTHTHGRKVTCH
QPSQALWSGILLVALGLIMLLM-TTVMAAVIGFIGVFTYVVLVTMWRKRTINTVVGSVGAVPLIGWAVEGNIGVAVNLFMILFIWQIPHFALALAKKTEDYRAANIPLMPDVYGFVEVTKRQ
APGEALGIGLALSGIAVVMLGLATNLFAGLLAFTIFFYAVVYSMWLKRRTQNIIVIGGAAGAFPPMIGWAVATGGVSVESLFFMFIWQIPHFALALFMKDDYSKAGVPMILTHTHGRVTRAH
SPAVSLVYATLLGIAGFMLLWFGANPLACWLGVMGFVVYGVSYLYMKRHSVYGTILGSLSGAAPPVIGYCAVTGEFDSGAAILLAIFSLWQMPHSYAIARFRKDYQAAIIPVLVVKGISVAKNH
              # # # # #
IW-YYSLLVVPFSLLLVYPLHQLGILYLAIAIILGGQFLVKAWQLKQAPGDRDL-----ARGLFKFSIFYLMLLCLAMVIDS-LPVTHQLVAQMGTLLLG 316
LRHCLALLVLSAAAP-VLDITTTWTFPIMPALPINAYISHL---GFRFYV-DADRRS-----SRRLFFCSLWHLPLLLMLLTC-KRPSGGGAGAPPPS 443
VSLRYSILMPLPCFGLSY-FNITDWYYQIDSLGILNA-WLTF-WAFKFYV-QQRINYSAKTLKDNVFNKGLSVANIYARKTFMASVLHL-PAIILAIHKKGRWDWIYPGEAKRPQERF 462
MVVYVAALL-PVSLML-YPF---GLVYTIIVAAVLGVGWLAL-GIAGFKMKDDIKW-----ARLMFVYSILNYLTIFVL-MVIVHF 284
IFA-YTLVLAPFALWLG-F-TSVGGPLYLAVSVNLALFIAGGWQILRRSEDQAQ-DGYRVEKRY-----FRLSLYYTFLHFLALLVQHVVGGW 279
IIVVWACL-M-PLPFLGS-LGLP-IVILGILLNIG-WLIL-GLMGFRSKNIMKWATQM-----FVYSLNYMTIYFVAMVLT-LF 305
VLVYS-LLLAPLAVAGAF-TGTGGPLYLATALALNG-WLLV-GAVRTWRRDEQAQAEADRYRVEKA-----FFRFSLYLFLHFGAILAEALKPYGLGGW 312
ITLYIIAFAVATLMLSLGGYAGYKYLVAASVWNLGMALRGY-KVA--DDRIV-----ARKLFGFSIIAITALSVMSVDFMVPDSTLLAAVW 296
              # $ # # #

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et al. [4], demonstrated that 23 amino-acid residues were of vital importance for the function of this enzyme. We found that 20 of these 23 amino-acid residues are conserved in the CoxD protein of *Synechocystis* sp. PCC 6803. Tyr-151 of CyoE is replaced by Trp in the CoxD of *Synechocystis* sp. PCC 6803 and in all the heme O synthases characterized to date. Lys-206 and Asp-257 of CyoE are shifted to the -2 and $+1$ positions, respectively, in the protein from *Synechocystis* sp. PCC 6803.

The CoxD protein exhibits the highest homology to the heme O synthases of *Rhodobacter sphaeroides* (28.7 SD) and *Paracoccus denitrificans* (22.5 SD), which were placed in the same taxonomic division by Woese [12], and we found that the homology score between these heme O synthases is 38.5 SD. The homology scores with respect to the heme O synthases of *Bacillus subtilis* (16.6 SD), *Bacillus firmus* (18.2 SD), and *E. coli* (14.0 SD) are also very significant. When we compared heme O synthases of *Rh. sphaeroides*, *P. denitrificans*, *Bacilli*, and *E. coli* to each other, we obtained lower values (e.g., *P. denitrificans* vs. *E. coli*, 9.5 SD; *P. denitrificans* vs. *B. subtilis*, 12.1 SD; and *B. subtilis* vs. *E. coli*, 13.2 SD). When we compared bacterial and mitochondrial heme O synthases [13,14], we found that the CoxD of *Synechocystis* sp. PCC 6803 gave homology scores of 10.4 SD and 12.6 SD when compared to yeast and human COX10 proteins, respectively, whereas the heme O synthases from other bacteria gave homology scores of 5.0–9.2 SD when compared to

the yeast protein and 7.5–11.7 SD when compared to the human protein.

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