**Communication**

**Primary Structure of Cytochrome \( b_5 \) from Cauliflower (Brassica oleracea L.) Deduced from Peptide and cDNA Sequences\(^1\)**

Ellen V. Kearns\(^2\), Pamela Keck, and Chris R. Somerville\(^*\)

Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824–1312 (E.V.K., C.R.S.); and Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198 (P.K.)

**ABSTRACT**

Cytochrome \( b_5 \) is a microsomal protein that functions as an intermediate electron donor in fatty acid desaturation and other oxidation/reduction reactions. cDNA clones were isolated from cauliflower (Brassica oleracea L.) by using oligonucleotides based on the partial amino acid sequence of the protein. The deduced amino acid sequence of the polypeptide exhibited approximately 30% sequence identity with the homologous protein from vertebrates.

Cyt \( b_5 \) is an integral membrane protein that is associated with the microsomal membranes of higher plants (3, 17) and animals (14). In animals, the protein has been implicated as an intermediate electron donor in fatty acid desaturation (9, 21), Cyt P-450 monoxygenase activity (13), cholesterol biosynthesis (16), fatty acid elongation (8), and plasmalogen biosynthesis (15). In higher plants, Cyt \( b_5 \) has recently been shown to function as an intermediate electron donor in the desaturation of fatty acids (7, 20) and has been suggested as a possible participant in \( \beta \)-oxidation in glycoxysomes (3). Considering the diversity of functions in animals, it seems possible that Cyt \( b_5 \) also has a role in other oxidation/reduction reactions in higher plants.

Previous investigations of the structure of Cyt \( b_5 \) from plants have resulted in purification of the protein to varying degrees from microsomal preparations of potato tubers (2), etiolated seedlings of Catharanthus roseus (10), etiolated pea stems (5), and cauliflower florets (6, 7). We report here the isolation and characterization of the first cDNA clone for Cyt \( b_5 \) from a plant. The availability of the cDNA clones should provide novel opportunities to experimentally examine the role of Cyt \( b_5 \) in other oxidation/reduction reactions in higher plants.

**MATERIALS AND METHODS**

Genetic Materials

*Escherichia coli* strain XL1-Blue was obtained from Stratagene (La Jolla, CA). A λUNI-ZAP XR cDNA library prepared from the meristematic surface of cauliflower floret (Brassica oleracea var cauliflora) was obtained from June Medford (12).

Peptide Sequence Analysis

Cyt \( b_5 \) was purified from cauliflower floret microsomes as previously described (7) and digested to completion with trypsin. The resulting peptides were resolved by HPLC on a C\(_{18}\) reversed-phase column and subjected to automated Edman degradation on a gas-phase sequenator.

Cloning Procedures

As a matter of convenience, a partial cDNA clone was first isolated from canola (Brassica napus L.), then used as a probe to isolate a full-length cDNA clone from *B. oleracea*. It was assumed, and subsequently verified empirically, that Cyt \( b_5 \) from the two species would share sufficient amino acid sequence identity so that mixed oligonucleotide probes based on *B. oleracea* peptide sequences would hybridize to cDNA from *B. napus*.

Single-stranded cDNA was prepared from 1 \( \mu \)g of total RNA from developing seeds of canola by priming the reverse transcriptase reaction with the mixed oligonucleotide 5′-TC(A/G)AT(C/T)TC(A/T)CC(A/G)ATGTA. The cDNA was then used as the template in a polymerase chain reaction (4) that was primed at the 3′ side with the mixed oligonucleotide above, and at the 5′ side with the mixed oligonucleotide 5′-GGNTT(C/T)GA(A/G)GA(A/G)GTNTC. The oligonucleotides were designed to encode the *B. oleracea* Cyt \( b_5 \) peptide sequences YYGEID and GFEVEVS, respectively. The relative orientation of these two peptides within the Cyt \( b_5 \) polypeptide was determined beforehand by alignment with animal
Cyt b$_5$ sequences (14). The 0.2 kilobase product of the polymerase chain reaction was cloned into the HindIII site of pUC119 and used to probe nitrocellulose filter replicas of a λUNI-ZAP XR cDNA library from cauliflower florets (11). The filters were hybridized at 65°C in 5 × SSPE (1 × SSPE = 150 mM NaCl, 10 mM Na$_2$HPO$_4$, 1 mM EDTA [pH 7.4]), 0.5% (w/v) nonfat dry milk, 0.5% (w/v) SDS, 5% (w/v) dextran sulfate, and washed twice at room temperature and once at 65°C in 2 × SSPE, 0.1% SDS. The positive clones were converted to pBluescript SK$^+$ form by coinfection with an M13 helper phage.

The DNA sequence of the cDNA inserts was determined by manual dideoxy sequencing of double-stranded plasmid DNA using synthetic oligonucleotides as primers.

**Structural Analyses**

Hydropathy plots were calculated using the algorithm of Rose and Roy (18) as implemented in the Hitachi PROSIS software package for microcomputers.

**RESULTS AND DISCUSSION**

The extra-membrane domain of Cyt b$_5$ from microsomal membranes of cauliflower florets was solubilized by treating the membranes with trypsin, and purified by column chromatography (7). The amino acid sequence of the amino terminus and several tryptic peptides was determined by gas-phase sequencing (Fig. 1). Degenerate oligonucleotides based on the partial amino acid sequence were then used in the polymerase chain reaction (4) to amplify a 220-base pair DNA fragment from *Brassica napus* cDNA. The product of this reaction was used as a hybridization probe to identify Cyt b$_5$ cDNA clones from a cauliflower cDNA library in λZAP. The DNA sequence of four of the largest cDNA clones was completely determined on both strands. The four sequences were in perfect agreement in the region of overlap except that one of the clones had a G-to-T transversion at nucleotide 110 (Fig. 1) that resulted in a lysine-to-asparagine change in the deduced amino acid sequence. One cDNA extended from nucleotide 1 to 585. Two cDNAs extended from nucleotide 68 to 675. These three cDNAs contained poly(A) tails of 19 A-residues at the 3′ end. The fourth cDNA extended from nucleotide 74 to 738 and lacked a poly(A) tail.

The cDNA clones encoded a polypeptide of 134 amino acids with a predicted molecular mass of 15 kD. For the peptides where amino acid sequence was obtained (underlined regions of Fig. 1), the deduced amino acid sequence was in perfect agreement with the directly determined sequences. The first five N-terminal amino acids of the deduced amino acid sequence were not present on the trypsin-solubilized polypeptide, suggesting that the peptide bond between the fifth and sixth residues was susceptible to cleavage by trypsin. Trypsin cleavage of Cyt b$_5$ from the ER of cauliflower florets was previously shown to result in a polypeptide of apparent molecular mass of about 12 kD on SDS-PAGE (7). This suggests that the trypsin cleavage occurred adjacent to lysine-110, which, in addition to cleavage at lysine-5, would produce a polypeptide of predicted molecular mass of 11 kD. However, in view of the potential error in molecular
mass estimates based on mobility in SDS-PAGE, cleavage at lysine-103 is also a possibility.

When aligned for maximum sequence identity but minimum displacement (Fig. 2), the B. oleracea polypeptide showed 31% sequence identity with the homologous protein from chicken (Gallus gallus) (22). This increased to 44% if conservative substitutions (i.e. Q/N, E/D, K/R, M/L/V/I, F/Y/W) were considered. The sequence identity was highest in the region of the protein surrounding the pairs of histidine residues (His-40 and His-64) that correspond to the axial ligands for heme binding by the protein from animals. Although the plant and animal amino acid sequences were divergent in the carboxyterminal third of the polypeptide, both polypeptides had a strikingly similar hydrophathy plot (Fig. 3). This apparent conservation of a region of higher order structure rather than sequence per se was previously described as an example of a "topogenic determinant" (1). Both proteins contained a strongly hydrophobic carboxyterminal domain to anchor the proteins in the membrane. The carboxy-terminal amino acid sequence of the B. oleracea protein (KKX) was reminiscent of the KXXX motif found on the cytoplasmically exposed carboxy terminus of certain proteins of the ER (19). This is consistent with the observation that the carboxy terminus of the vertebrate Cyt b5 has been shown to be located on the cytoplasmic side of the ER (14). Surprisingly, there was no sequence identity of the cauliflower polypeptide with an amino-terminal amino acid sequence previously reported for a Cyt b5 from pea (Pisum sativum) (5).

The cDNA clone described here is the first Cyt b5 gene characterized from a nonvertebrate species. The availability of the clone should create many new opportunities to investigate the involvement of this protein in microsomal oxidation and reduction reactions in higher plants.

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