A Subunit of Mammalian Signal Peptidase Is Homologous to Yeast SEC11 Protein*

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Canine signal peptidase consists of a complex of five proteins (Evans, A. E., Gilmore, R., and Blobel, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 581–585). A cDNA encoding the 21-kDa subunit of the signal peptidase complex was isolated from a liver cDNA library using an 88-base pair probe, generated by the polymerase chain reaction. The 820-base pair cDNA was sequenced and found to encode a protein of 21,585 daltons. The deduced amino acid sequence from the canine cDNA was found to be 47% identical to the yeast SEC11 protein. SEC11 has been shown to be required for signal peptide cleavage and cell survival in Saccharomyces cerevisiae (Bohni, P. C., Deshaies, R. J., and Schekman, R. W. (1988) J. Cell Biol. 108, 1035–1042). It is, therefore, likely that the 21-kDa subunit of signal peptidase complex is the structural and functional homologue of the yeast SEC11 gene product.

Signal sequences of bacterial, yeast, and mammalian presecretory proteins are similar both structurally and functionally. This similarity permits translocation of presecretory proteins across the prokaryotic plasma membrane or the endoplasmic reticulum of yeast and mammals in an interchangeable manner (1–4). Likewise, the signal peptide associated with the prokaryotic plasma membrane or the eukaryotic endoplasmic reticulum can cleave signal sequences of presecretory proteins from diverse species (1, 5, 6). Although several components of the prokaryotic and eukaryotic translocation apparatus as well as the corresponding signal peptidases have been isolated and characterized, structural homologies of components of the translocation apparatus or of the signal peptidases between prokaryote, yeast, or higher eukaryotes have so far not been reported.

Canine signal peptidase has been purified to near homogeneity from salt-extracted and detergent-solubilized pancreatic microsomes (7). It consists of a complex of five proteins (molecular mass of 12, 18, 21, 23, and 25 kDa) (7). The conclusion that the pancreatic enzyme is a pentameric complex rests on two lines of evidence. First, the detergent-solubilized enzyme complex remained associated throughout the purification procedure including sucrose gradient sedimentation (7). Second, the pentameric complex could be isolated by concanavalin A-Sepharose affinity chromatography, although only one of its subunits (23 kDa) is core-glycosylated (9). Recently the cDNA for the 23-kDa glycoprotein subunit of the canine SPC has been cloned and sequenced (10). The deduced amino acid sequence showed no sequence homology to proteins in the GenBank and Dayhoff data bases.

Unlike canine signal peptidase, hen oviduct signal peptidase has been reported to consist of only two subunits, a glycoprotein subunit of 22–24 kDa and a nonglycosylated subunit of 19 kDa (11). Signal peptidase I (leader peptidase) of Escherichia coli, the functional equivalent of the eukaryotic signal peptidase consists of only one polypeptide of Mr = 35,994 (12).

Here, we describe the cloning and sequencing of a CDNA encoding the 21-kDa subunit of the canine signal peptidase complex and find it to be highly homologous to the yeast SEC11 gene product, which has been shown genetically to be required for signal peptide cleavage and cell survival (8).

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes, pUC 19 vector, and Random Primed DNA labeling kit were from Boehringer Mannheim. T4 polynucleotide kinase, T4 DNA polymerase, and T4 DNA ligase were from New England Biolabs. Avian myeloblastosis virus reverse transcriptase was obtained from Life Sciences Inc. Dideoxy sequencing was performed using modified T7 DNA polymerase (Sequenase 2.0) and reagents from United States Biochemical Corp. The compounds [α-32P]deoxycytidine 5'-triphosphate and [γ-32P]deoxyadenosine 5'-triphosphate were from Du Pont-New England Nuclear. Taq DNA polymerase, deoxynucleotides, and buffer used in the polymerase chain reaction were from Perkin-Elmer-Cetus (GeneAmp kit N801-0043).

Protein Sequencing—The 21-kDa protein was purified from canine pancreas as described previously (7, 10). For amino-terminal sequencing the purified protein was applied in 0.5% sodium dodecyl sulfate directly to an Applied Biosystems model 470A gas-phase sequenator (13). For internal amino acid sequence, the purified protein was first cleaved with CNBr (14) and the cleaved peptides separated by reverse phase high performance liquid chromatography using a C-4 column in an H2O/acetonitrile gradient containing 0.1% trifluoroacetic acid.

Polyonymerase Chain Reaction—The nucleotide probe used to isolate clones was generated by PCR (15) using Taq polymerase. Two oligonucleotides corresponding to the extreme ends of the amino-terminal protein sequence were synthesized with restriction sites using an Applied Biosystems model 380B oligonucleotide synthesizer. 0.5 μM of gel-purified oligonucleotide 1, 5'-CGCCTAGGCGGCGCGGTGCGGAXCXA-3', and oligonucleotide 2, 5'-CXTTTTTTACTTTTTCTTTAAGCG-3', were used in the PCR with template cDNA synthesized from canine liver RNA (16), which was poly(A)+-selected using oligo(dT)-cellulose chromatography (17). The reaction was run in a DNA thermal cycler (Perkin-Elmer-Cetus) with the following incubation conditions: denaturation at 95 °C for 1 min,
annaling at 45 °C for 2 min, and extension at 72 °C for 3 min, with reaction component concentrations described by Perkin-Elmer Cetus. The PCR product (20C) was cloned into the BamHI and EcoRI cloning site of pUC 19 and sequenced.

cDNA Library Screening—The cDNA clone 4AA was isolated from an amplified Agt10 canine liver library (10). The 20C product was labeled with [α-32P]dCTP (Du Pont-New England Nuclear NE-931H) using the random-primed method (18). Plaque lifts were hybridized with the 32P-labeled 20C product, and positive plaques were isolated and placed in PCR buffer (50 mM KCI, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 0.01% (w/v) gelatin). 50 μl of eluted phage was used as template in a PCR to identify inserts near the transcript size of 1 kilobase. The PCR contained oligonucleotide 1 (above) and one of two primers flanking the EcoRI restriction site of Agt10 (New England Biolabs 1231 or 1232). An amplification product of 0.85 kilobase was identified, and the corresponding plaque was selected for re-plating and three more rounds of filter hybridization were performed. The insert from plaque-purified clone 4AA was subcloned into the EcoRI site of pUC 19 and sequenced.

DNA Sequencing—The double-stranded template was sequenced using the disoxy chain termination method of Sanger et al. (19) using synthetic oligonucleotides (20). Both strands of the double-stranded template were sequenced with Sequenase (United States Biochemical Corp.) using both dGTP and dITP. Searches of GenBank and DDBJ databases were performed on the Rockefeller University VAX computer using FASTA (21). Alignment was performed using the method of Needleman and Wunsch (22), as implemented on the DNAstar AALIGN program (DNAstar Inc., Madison, WI) on an IBM AT computer.

RESULTS AND DISCUSSION

To clone the cDNA for the 21-kDa subunit of SPC, we synthesized degenerate oligonucleotides based on information derived from the amino acid sequence of the NH2-terminus of the 21-kDa subunit. We used these in a PCR (15) to obtain a DNA probe of 88 base pairs which was used to screen a cloned canine liver cDNA library in Agt10. The cDNA sequence of one clone is shown in Fig. 1. It contains a single open reading frame of 576 base pairs, revealing a protein of 192 amino acids and a calculated molecular mass of 21,585 daltons, in good agreement with the Mr = 21,000 estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7). The clone begins with a 208-nucleotide 5′-untranslated region. There is a Kozak consensus sequence (CCGCACTAGG) surrounding the initiating ATG codon (23) of the open reading frame. This clone (4AA) contains a total of 820 base pairs, close to the transcript size of 1 kilobase determined by Northern blot analysis (data not shown). No polyadenylation signal was found, probably due to the short 3′-untranslated region (36 nucleotides) of the clone. The deduced amino acid sequence of the 21-kDa clone was confirmed by NH2-terminal and internal amino acid sequence data obtained by automated Edman degradation of purified 21-kDa protein. Met1 of the cDNA deduced amino acid sequence is followed by a Val residue which is the NH2-terminal residue of sequence derived from Edman degradation. Thus, the NH2-terminal Met in the primary translation product has been removed, as is seen for many proteins where Met is followed by Val (24). Another putative initiation codon upstream (nucleotide position −99) is out of frame. Thus, it is clear that the 21-kDa protein is not synthesized with a cleavable signal sequence.

A homology search comparing the 21-kDa deduced amino acid sequence with sequences present in the GenBank and DDBJ nucleotide databases was negative. Comparison of the deduced amino acid sequence of the 21-kDa subunit of SPC with the recently published sequence of the yeast SEC11 protein (8) revealed a striking sequence similarity (Fig. 2). From Met25 to Gly25 the 21-kDa protein is 47% identical to the SEC11 protein. A 30-residue stretch within this region (Thr45-Arg50) is 81% identical. The high degree of structural homology in this region suggests conservation of an important functional aspect that is shared by the 21-kDa and SEC11 proteins.

The average hydrophy of the deduced amino acid sequence was plotted as described by Kyte and Doolittle (25) (Fig. 3). A large hydrophobic domain is located in the first half of the protein between residues 29 and 69 (Fig. 1). 20 amino acids of this hydrophobic domain (Fig. 1, residues 29–48) were bordered by charged residues at positions 28 and 49. This region has an average hydrophy value of 1.6, making it a possible candidate for a transmembrane domain (25) and an internal uncleaved signal sequence. This domain is followed by a very hydrophilic region that comprises more than a third of the protein. The last portion of the protein (Fig. 1, residues 157–168) is relatively hydrophobic but contains less than the 20 amino acids needed to span the lipid bilayer. The two hydrophobic domains of the 21-kDa protein correspond closely to those of the SEC11 protein (8). Interestingly, like the 21-kDa and SEC11 proteins, the 23-kDa protein of the SEC also contains two hydrophobic domains (10). The first is thought to be a membrane anchor while the second resides in the lumen of the endoplasmic reticulum. The fact that bacterial signal peptides also contain a hydrophobic domain in the periplasm (26) (which corresponds to the endoplasmic reticulum lumen) suggests that this structural motif might be involved in a common function necessary for signal peptide cleavage.

As separation of the subunits of the SPC without denaturation has been unsuccessful (9) the protein subunit(s) responsible for signal peptide cleavage have yet to be identified. Genetic analysis of yeast mutants has clearly demonstrated that the SEC11 protein is required for cell survival and signal
The homology of the canine 21-kDa protein and yeast SEC11 protein. The two sequences are 47% identical over the 167 amino acids of the overlapping sequences. The deduced amino acid sequence of the 21-kDa protein is shown on the top, and the SEC11 gene product, which begins at amino acid 25 of the 21-kDa protein, on the bottom. Regions of amino acid identity are enclosed by boxes. Gaps in the sequence are indicated by a dash.

Peptide processing (8). It is likely that the 21-kDa canine homologue of SEC11 protein has a similar if not identical function in the mammalian endoplasmic reticulum. Whether these two proteins are the catalytic subunit of signal peptidase remains to be determined, but the lack of sequence homology with the larger bacterial signal peptidase I (12) suggests that another protein may contain the signal sequence cleavage activity, while these proteins function in an ancillary way (8).

The recent evidence that SEC11 protein is a member of a protein complex with signal peptidase activity2 taken together with the work presented in this paper raises the intriguing possibility that, like the 21-kDa protein, other members of the canine SPC may have counterparts in yeast. Expression of the canine 21-kDa protein and the other subunits of the canine SPC in yeast and in yeast secretory mutants may help to elucidate their role in signal sequence processing and protein translocation.

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