Membrane Topology of the 12- and the 25-kDa Subunits of the Mammalian Signal Peptidase Complex*

(Received for publication, September 22, 1995, and in revised form, November 28, 1995)

Kai-Uwe Kalies‡ and Enno Hartmann§
From the Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, 13125 Berlin-Buch, Germany

The cleavage of signal sequences of secretory and membrane proteins by the signal peptidase complex occurs in the lumen of the endoplasmic reticulum. Mammalian signal peptidase consists of five subunits. Four have been cloned, SPC18, SPC21, SPC22/23, and SPC25, of which all but SPC25 have been demonstrated to be single-spanning membrane proteins exposed to the lumen of the endoplasmic reticulum.

We have determined the cDNA sequence of the remaining 12-kDa subunit (SPC12) as well as the membrane topologies of SPC12 and SPC25 in rough microsomes. Both polypeptides span the membrane twice with their N and C termini facing the cytosol and contain only very small, if any, luminal domains. Therefore, SPC12 and SPC25 are likely to be involved in processes other than the enzymatic cleavage of the signal sequence.

During the next step, translocation of the nascent chain through the membrane, the signal sequence of most secretory and membrane proteins is cleaved off. Cleavage occurs by the signal peptidase complex (SPC) as soon as the luminal domain of the translocating polypeptide is large enough to expose its cleavage site to the enzyme (Blobel and Dobberstein, 1975b). The signal peptidase complex is possibly also involved in proteolytic events in the ER membrane other than the processing of the signal sequence, for example the further digestion of the cleaved signal peptide (Lyko et al., 1995) or the degradation of membrane proteins (Mullins et al., 1995).

Mammalian signal peptidase has been purified from dog pancreas microsomes as a complex of five different polypeptide chains (Evans et al., 1986). The cDNAs of four of the subunits (SPC18, SPC21, SPC22/23, SPC25) have been cloned and sequenced (Shelness et al., 1988; Greenburg et al., 1989; Greenburg and Blobel, 1994). SPC18 and SPC21 are single-spanning membrane proteins; the majority of both proteins is located within the lumen of the ER and contains a second only moderately hydrophobic sequence (Shelness et al., 1993). Both subunits show high homology to each other (Shelness et al., 1988). Moreover, they are related in sequence to leader peptidase (van Dijl et al., 1992), the enzyme responsible for signal sequence cleavage during translocation of proteins across the plasma membrane of bacteria (Zwizinski and Wickner, 1980). These polypeptides may therefore function as catalytic subunits. SPC22/23 has an identical topology to SPC18 and SPC21. It is also a single-spanning membrane protein with a second only somewhat hydrophobic segment located in the lumen of the ER (Shelness et al., 1993). SPC22/23 contains an N-glycosylation site, and its migration in SDS-gels as two bands of 22 and 23 kDa, respectively, is likely to arise from differential trimming of its oligosaccharide moiety (Evans et al., 1986). SPC25 has recently been cloned. It again contains two hydrophobic sequences and was proposed to be a single-spanning membrane protein as well, with a large N-terminal domain in the lumen of the ER (Greenburg and Blobel, 1994). However, its membrane topology has not yet been directly determined.

In this paper, we present the sequence of the remaining subunit of the mammalian signal peptidase complex (SPC12) as well as the membrane topologies of SPC12 and SPC25. Surprisingly, both polypeptides are largely exposed to the cytosolic compartment and have very few, if any, amino acid residues in the lumen of the ER. Since this is where the active site of the enzyme must be located, SPC12 and SPC25 are not likely to be directly involved in signal sequence cleavage.

EXPERIMENTAL PROCEDURES

Materials—DNA purification kit was purchased from QIAGEN, proteinase K from Boehringer Mannheim, Taq DNA polymerase and DNA sequencing system from Promega, and DNA modifying enzymes from New England Biolabs or Boehringer Mannheim. [γ-32P]ATP was from Amersham. Keyhole limpet hemocyanine and sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) were used from Pierce.

Antibodies—Antibodies against the N terminus of TRAPβ and the C terminus of SPC22/23 have been described (Görlach et al., 1990; Vogel et al., 1990). Anti SPC12 antibodies were made against an N-terminal...
peptide (MLEHLSSLPTQMDYKC) and anti-SPC25 antibodies against a C-terminal peptide (CHDSLATERKIK), respectively. The peptides were coupled to keyhole limpet hemocyanine that had been activated with sulfo-SMCC. The immunization of rabbits as well as the affinity purification were performed as described (Görlich et al., 1992).

**RESULTS**

**Sequence of SPC12**—The signal peptidase complex was purified from canine microsomes as described. The 12-kDa polypeptide was isolated, and partial amino acid sequences were obtained. Degenerate oligonucleotides were designed and used to clone the entire coding region of the gene from a human fetal brain cDNA library. The nucleotide sequence and the derived amino acid sequence, which contains all the directly determined peptide sequences, are shown in Fig. 1. The correct assignment of the N terminus of the protein is supported by N-terminal peptide sequence data and by the fact that the upstream region contains no other start codons. Mismatches between the canine peptides and the human protein are probably due to species differences. The predicted size of the protein (11,810 Da) corresponds very well to that determined by SDS-PAGE. The sequence shows no similarity to any of the other four signal peptidase subunits cloned previously and does not share any obvious homology with other protein sequences in the database except for several "unidentified" sequence tags from human cDNA libraries and for a sequence tag from rat (accession number H35785). Like the other signal peptidase subunits, SPC12 does not have a cleavable signal sequence.
The polypeptide contains two hydrophobic segments between residues 26–47 and 49–69 (Fig. 1). Sequence analysis with computer programs using different algorithms (SOAP, Klein et al. (1985); RAOARGOS, Rao and Argos (1986); HELIXMEM, Eisenberg et al. (1984)) predicts that both hydrophobic regions have a high probability of functioning as transmembrane segments. Moreover, according to the charge distribution between the flanking regions of the membrane anchors, it is likely that the N and C termini of the protein face the cytoplasm (Hartmann et al., 1989).

Membrane Topology of SPC12—To determine the membrane topology of SPC12, ribosome-stripped canine pancreatic microsomes were treated with proteinase K, and digestion of the protein was followed by immunoblot analysis. The latter was carried out with affinity-purified antibodies raised against the N terminus of SPC12 (Fig. 1). These antibodies recognized a single protein of the expected size in dog pancreatic microsomes (Fig. 2, lane 1).

If microsomes were treated in the cold with proteinase K, SPC12 migrated in SDS gels with an apparent molecular mass of 11 kDa, in contrast to 12 kDa for the undigested protein, and showed an unchanged reactivity toward the antibody (Fig. 2, lane 2). The same results were observed when trypsin was used instead of proteinase K (data not shown). The N-terminal antigenic peptide of SPC12 does not contain a trypsin cleavage site. For all of these reasons, it is clear that a portion of the C terminus was cut off, and thus this region must be located in the cytosol, accessible to the protease. Surprisingly, if the digestion was carried out after solubilization of the microsomes in Triton X-100, no further degradation was observed (Fig. 2, lane 3). A control protein, TRAPβ (originally called SSRβ), a single-spanning membrane protein with only few C-terminal residues located in the cytosol (Hartmann et al., 1993), did not change significantly in size after proteinase K treatment but was completely digested if Triton X-100 was also added (lanes 2 and 3). On the other hand, SPC22/23 behaved similarly to SPC12 in that its accessibility to proteases was the same before and after solubilization of the membranes. In both cases, the mobility of SPC22/23 was unchanged after protease digestion (lanes 2 and 3). The remarkable protease resistance of SPC18, SPC21, and SPC22/23 has been reported before (Shelness et al., 1993). Therefore, it seems that the signal peptidase is a stable complex of polypeptide chains remaining almost resistant to protease treatment even after solubilization of the membranes.

We reasoned whether this unusual behavior could be exploited to determine the localization of the N terminus of SPC12, which is recognized by the antibody. We pretreated microsomes at elevated temperatures with the hope that the tight interaction between the signal peptidase subunits can be broken by heat. The subsequent protease digestion was carried out on ice in the absence or presence of Triton X-100. As shown in Fig. 2 (lanes 6–9), pretreatment at 50 or 60°C allowed the complete degradation of the N terminus of SPC12, even in the absence of detergent. In contrast, TRAPβ and SPC22/23 were only digested in the presence of Triton X-100, demonstrating that these proteins have maintained their membrane orientation with a major luminal domain and that the membrane integrity was not compromised by the heat treatment. These data confirm the membrane topologies of TRAPβ and SPC22/23 and show that both the N and C termini of SPC12 are located in the cytoplasmic compartment. Such a model is in good agreement with the predicted membrane topology of SPC12 having two adjacent membrane-spanning segments.

Fig. 3. Sequence of canine SPC25.

The dog amino acid sequence is shown (Greenburg and Blobel, 1994). Proposed membrane anchors are double underlined. Peptides used to raise antibodies are indicated with asterisks above.

**Table 1: Accessibility of SPC12 and SPC25 to proteases.** Ribosome-stripped microsomes were incubated at the indicated temperatures for 15 min and then subsequently cooled down to 0°C. Proteolysis was carried out with 250 μg/ml proteinase K for 1 h on ice. As indicated, 1% Triton X-100 was added before starting proteolysis. The samples were precipitated with 20% trichloroacetic acid and analyzed by SDS-PAGE and immunoblotting. The following peptide-specific antibodies were used: TRAPβ, N terminus; SPC22/23, C terminus; SPC25, C terminus; SPC12, N terminus. The stars indicate degradation products of TRAPβ.
The mammalian signal peptidase complex consists of five subunits (Evans et al., 1986), and, with the data on SPC12 and SPC25 presented here, the sequences and membrane topologies of all subunits are now known (Fig. 4). The primary structure of SPC12 is not related to any of the other four signal peptidase subunits (SPC18, SPC21, SPC22/23, and SPC25) and shows no homology to any known protein in the data bases. SPC12 contains two hydrophobic segments close to each other. As in the case of SPC25, the hydrophobicity of either segment is substantially higher than that of the second somewhat hydrophobic regions found in SPC18, SPC21, and SPC22/23. This suggested to us that SPC12 and SPC25 must span the membrane twice. To test this, dog pancreatic microsomes were treated with proteinase K, and the degradation of both polypeptides was followed using affinity-purified peptide-specific antibodies. Indeed, SPC12 and SPC25 each contain two adjacent membrane anchors with both their N and C termini oriented toward the cytosolic compartment (Fig. 2). Surprisingly, both subunits expose only very few, if any, amino acids to the lumen of the ER where the catalytic site of the signal peptidase complex must be positioned. Our data clearly contradict the recent assumption that SPC25 is a single-spanning membrane protein with an extended N-terminal segment inside the ER lumen (Greenburg and Blobel, 1994). The reported disulfide bridge in the native protein (Greenburg and Blobel, 1994) could be a linkage between two of the three cysteins present in the membrane anchors as has been found in the leader peptidase of the Escherichia coli plasma membrane (Whitley et al., 1993).

Interestingly, SPC12 and SPC25 remained almost protease resistant even when the membranes were solubilized with detergent before adding the protease. The same phenomenon has previously been described for SPC18, SPC21, and SPC22/23 (Shelness et al., 1993). Thus, the five polypeptides of the mammalian signal peptidase seem to form a very tight complex whose core is resistant to protease treatment even after solubilization of the membrane. Our results further indicate that the compact structure of the signal peptidase complex can be broken by heat treatment. After incubation of the microsomes at 50 or 60 °C, additional regions of SPC12, SPC22/23, and SPC25 became accessible to the added protease. Heat pretreatment of the microsomes did not influence the membrane orientation of the two control proteins, SPC22/23 and TRAPβ. These data are in good agreement with results of other groups. Fujimoto et al. (1984) had found that an incubation of partially purified SPC at 40 °C slightly decreased its enzyme activity and that further increase of the temperature inactivated the enzyme. Shelness et al. (1993) observed that SPC18, SPC20, and SPC22/23 were more accessible to proteases after treating a detergent extract of microsomes at 75 °C.

What might be the functions of the different signal sequence subunits? Because of their homology to the leader peptidase of bacteria, SPC18 and SPC21 may function as catalytic subunits and are believed to represent members of a novel protease family, which have a serine as a key amino acid in the active site (van Dijl et al., 1992; Dalbey and von Heijne, 1992; van Dijl et al., 1995). The membrane topologies of SPC18 and SPC21 fit with this hypothesis as the majority of both polypeptides, including the proposed catalytic center, are exposed to the lumen of the ER (Shelness et al., 1993). Even though SPC22/23 has the same membrane orientation, a direct contribution to signal sequence cleavage seems unlikely as it has no homology to known proteases. However, it is possible that this polypeptide represents a new protease with an unknown substrate specificity. Alternatively, it has been speculated that SPC22/23 may be involved in substrate binding (Shelness et al., 1988).

ACKNOWLEDGMENTS—We thank Dirk Görlich for the purification of canine SPC, Susanne Kostka for protein sequencing, and Kristen J. Verhey and Tom A. Rapoport for critical reading of the manuscript.

REFERENCES

Bblobel, G., and Dobberstein, B. (1975a) J. Cell Biol. 67, 835–851
Bblobel, G., and Dobberstein, B. (1975b) J. Cell Biol. 67, 852–862
Dalbey, R. E., and von Heijne, G. (1992) Trends Biochem. Sci. 17, 474–478
Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. (1984) J. Mol. Biol. 179, 125–142
Evans, E. A., Gilmore, R., and Blobel, G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 581–585
Feldheim, D., and Schekman, R. (1994) J. Cell Biol. 123, 935–943
Fujimoto, Y., Watanabe, Y., Uchida, M., and Ozaki, M. (1984) J. Biochem. (Tokyo) 96, 1125–1131
Gilmore, R., Walter, P., and Blobel, G. (1982) J. Cell Biol. 95, 470–477
Görlich, D., and Rapoport, T. A. (1993) Cell 75, 615–630
Görlich, D., Prenh, S., Hartmann, E., Herz, J., Otto, A., Kraft, R., Wiedemann, M., Knespel, S., Dobberstein, B., and Rapoport, T. A. (1990) Cell Biol. 111, 2283–2294
Görlich, D., Prenh, S., Hartmann, E., Kelle, K.-U., and Rapoport, T. A. (1992) Cell 71, 489–503
Greenburg, G., and Blobel, G. (1994) J. Biol. Chem. 269, 25345–25358
Greenburg, G., Shelness, G. S., and Blobel, G. (1989) J. Biol. Chem. 264, 15762–15765
Hartmann, E., Rapoport, T. A., and Lodish, H. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5786–5790
Hartmann, E., Görlich, D., Kostka, S., Otto, A., Kraft, R., Knespel, S., Bürger, E.,
Membrane Topology of SPC12 and SPC25

Rapoport, T. A., and Prehn, S. (1993) Eur. J. Biochem. 214, 375–381
Johnsson, N., and Varshavsky, A. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 10340–10344
Jungnickel, B., and Rapoport, T. A. (1995) Cell 82, 261–270
Klein, P., Kanehisa, M., and DeLisi, C. (1985) Biochim. Biophys. Acta 815, 468–476
Lyko, F., Martoglio, B., Jungnickel, B., Rapoport, T. A., and Dobberstein, B. (1995) J. Biol. Chem. 270, 19873–19878
Meyer, D. I., Krause, E., and Dobberstein, B. (1982) Nature 297, 647–650
Mullins, C., Lu, Y., Campbell, A., Fang, H., and Green, N. (1995) J. Biol. Chem. 270, 17139–17147
Rao, J. K., and Argos, A. (1986) Biochim. Biophys. Acta 869, 197–214
Shelness, G. S., Kanwar, Y. S., and Blobel, G. (1988) J. Biol. Chem. 263, 17063–17070
Shelness, G. S., Lin, L., and Nicchitta, C. V. (1993) J. Biol. Chem. 268, 5201–5208
van Dijl, J. M., de Jong, A., Vehmanen, J., Venema, G., and Bron, S. (1992) EMBO J. 11, 2619–2626
van Dijl, J. M., de Jong, A., Venema, G., and Bron, S. (1995) J. Biol. Chem. 270, 3611–3618
Vogel, F., Hartmann, E., Görlich, D., and Rapoport, T. A. (1990) Eur. J. Cell Biol. 53, 197–202
Walter, P., and Blobel, G. (1983) Methods Enzymol. 96, 84–93
Walter, P., and Johnson, A. E. (1994) Annu. Rev. Cell Biol. 10, 87–119
Walter, P., Ibrahim, J., and Blobel, G. (1981) J. Cell Biol. 91, 545–550
Whitley, P., Nilsson, L., and von Heijne, G. (1993) Biochemistry 32, 8534–8539
Zwizinski, C., and Wickner, W. (1980) J. Biol. Chem. 255, 7973–7977
Membrane Topology of the 12- and the 25-kDa Subunits of the Mammalian Signal Peptidase Complex
Kai-Uwe Kalies and Enno Hartmann

J. Biol. Chem. 1996, 271:3925-3929.
doi: 10.1074/jbc.271.7.3925

Access the most updated version of this article at http://www.jbc.org/content/271/7/3925

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 17 of which can be accessed free at http://www.jbc.org/content/271/7/3925.full.html#ref-list-1