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Solid-state NMR investigations of peptide–lipid interactions of the transmembrane domain of a plant-derived protein, Hcf106

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ABSTRACT

The chloroplast twin arginine translocation system transports highly folded precursor proteins across the thylakoid using the protonmotive force as its only energy source. Hcf106 and another thylakoid protein, cpTatC compose the precursor receptor complex. Hcf106 is predicted to contain a single amino terminal transmembrane domain (TMD) followed by a Pro–Gly hinge, an amphipathic α-helix, and a loosely structured carboxyl terminus. Hcf106 has been shown biochemically to insert spontaneously into thylakoid membranes; however, how this occurs is not understood. To investigate how Hcf106 inserts itself into the membrane unassisted, solid-state NMR spectroscopy was used to investigate the membrane activity of the TMD. A synthetic peptide of the Hcf106 TMD was incorporated into multilamellar vesicles made of 100% 1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine (POPC) or 85%:15% ratio with monogalactosyl diacylglycerol (POPC/MGDG) to probe peptide–lipid interaction. Solid-state ^{31}P NMR and ^{2}H NMR spectroscopic techniques were used to reveal peptide perturbations of the phospholipid membranes. Changes in spectral lineshape, chemical shift anisotropy width, ^{31}P T_1 relaxation time and S_{CD} order parameters demonstrated that the Hcf106 TMD peptide interacted with the phospholipids. Furthermore, the comparison between POPC and POPC/MGDG multilamellar vesicles indicated that lipid bilayer composition affected the peptide–lipid interaction with the peptide interacting preferentially with vesicles that more closely mimic the thylakoid.

1. Introduction

In plant cells the genes for thylakoid lumen proteins are found in the nuclear genome. They are synthesized in the cytosol from mRNA as higher molecular weight precursors containing targeting sequences, which direct the protein to chloroplasts and subsequent localization to thylakoid lumen for function (Cline and Theg, 2007). There are two thylakoid transport systems, homologous to bacterial export systems that route precursors to the thylakoid lumen: the chloroplast Sec and Tat pathways (Cline and Dabney-Smith, 2008). The chloroplast twin arginine translocation (cpTat) system is unique in that it delivers highly folded proteins across the membrane utilizing only the trans-thylakoidal protonmotive force as its energy source. Precursors destined for the thylakoid lumen contain lumen targeting signal sequences and those that use the cpTat pathway contain an obligate twin arginine amino acid motif giving the pathway its name. The cpTat pathway is composed of three membrane-bound subunits, Tha4, Hcf106, and cpTatC (Celedon and Cline, 2012). Sequence analysis predicts that Tha4 and Hcf106 have similar structure, both consisting of an N-terminal transmembrane domain (TMD), followed by an amphipathic helix (APH) and an unstructured C-terminus (C-tail), yet the two proteins are functionally distinct because a lack of either protein cannot be compensated by the presence of the other, even in excess amounts (Fincher et al., 2003). The third protein, cpTatC, is an integral membrane protein with six transmembrane-spanning helices (Mori et al., 2001; Rollauer et al., 2012). cpTatC and Hcf106 form the receptor for signal peptide binding (Gérard and Cline, 2007; Ma and Cline, 2013), whereas oligomers of Tha4 assemble with the precursor-bound receptor complex in the presence of the trans-thylakoidal protonmotive force (Dabney-Smith and Cline, 2009; Pal et al., 2013). Like the precursors that they transport, the three cpTat translocation complex proteins are also nuclear encoded and thus contain targeting sequences that promote their proper plastid localization. For example, import and routing of cpTatC occurs via a stromal intermediate (Martin et al., 2009), and it cannot integrate into the thylakoid membrane directly. Conversely, Tha4 and Hcf106 do not appear to use a stromal intermediate, and they can spontaneously insert into thylakoid membranes even in the absence of their signal peptide (Fincher et al., 2003). Currently how these proteins are able to integrate directly into membranes unassisted is

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unknown. Here we investigated interactions of a synthetic peptide corresponding to the TMD region of Hcf106 with multilamellar vesicles (MLVs) containing phospholipids or a mix of phospholipids and monogalactosyl diacylglycerol. In addition, understanding the interaction of Hcf106 with lipid bilayers may provide insights into Hcf106 function and activity.

Solid-state NMR spectroscopy provides a precise way to study the integration, topology, and structure-function relationships of small membrane proteins or peptides and their interaction with lipids (Abu-Baker and Lorigan, 2006; Cross and Opella, 1994; Nakazawa and Asakura, 2003; Watts, 1998). Natural ^{31}P-containing and ^{2}H-labeled lipids are useful probes to study peptide-lipid interactions from the perspective of lipids. For example, ^{31}P nuclei located on the lipid surface are sensitive to the conformation of the lipid head group, phase, and electrostatic properties, and have been used extensively to investigate the dynamics and interaction between lipid head groups with peptides (Dave et al., 2004a; Gehman and Separovic, 2011; Ouellet et al., 2010; Santos et al., 2004; Smith and Ekiel, 1984). Complementary to ^{31}P NMR, ^{2}H solid-state NMR spectroscopy provides information on the orientation and dynamics of the acyl chains of lipids as peptides or proteins incorporate into deuterated acyl-chain phospholipid bilayers (Gehman and Separovic, 2011; Koenig et al., 1999; Lu et al., 2005; Ouellet et al., 2010; Strandberg et al., 2004; Yamaguchi et al., 2001). Often, however, peptide–lipid interactions are based not only on the amino acid sequence of proteins but also on the composition of lipids. Altering the lipid content can promote understanding of protein and lipid structure, hydrophobicity and aggregation of proteins, charge distribution on lipid, depth of peptide in the lipid milieu, and modulation of the peptide–lipid interaction (Arora and Tamm, 2001; Bloom et al., 1991; Lemmon and Engelman, 1994; Watts, 1981).

Solid-state NMR spectroscopy is used in this study to investigate the topology of vesicles from the perspective of the lipids, and to obtain preliminary topology information of Hcf106 in lipids by studying the peptide–lipid interaction. To simplify the study of this 176 amino acid protein, a peptide encompassing the TMD (residues 2–27) of Hcf106 was synthesized by Fmoc-based solid-phase methods, and purified by high-performance liquid chromatography (Tiburu et al., 2003). The peptide was then incorporated into POPC (1-palmitoyl-2-oleoyl-sn-glycero-phosphoatidylcholine) and POPC/MGDG (monogalactosyl diacylglycerol) vesicles at various concentrations. MGDG is a natural plant thylakoid lipid composing more than 50% of the thylakoid lipids. Its head group consists of a galactosyl group, lacking phosphates, and it is a native non-bilayer forming lipid. The synthetic phospholipid bilayers mimicked biological membranes providing a controlled environment in which to assay peptide interaction or insertion with the membrane. ^{31}P solid-state NMR spectroscopic data indicated that Hcf106 TMD peptides interact with phospholipid head groups. In addition ^{2}H NMR spectroscopic measurements (e.g., ^{2}H NMR order parameter) revealed the effect on the deuterated acyl chains by the presence of the peptide and the effect of lipid composition on the peptide–lipid interaction. These experimental results confirmed the membrane-association activity of the proposed transmembrane domain of Hcf106 and suggested that the Hcf106 TMD peptide interacts with the lipid head group region and is imbedded in the hydrophobic region of the lipid bilayer.

2. Experimental methods

2.1. Materials

Synthetic phospholipids POPC (MW 760.08) and POPC-d_{31} (MW 791.27) were purchased from Avanti Polar Lipids (Alabaster, AL). Natural galactolipid from parsley leaves, MGDG (approximate MW 778; predominant fatty acyl chain 16:3–18:3), was purchased from Larodan Fine Chemicals AB (Sweden, http://www.larodan.se). All lipids were dissolved in chloroform or chloroform/methanol and stored at −20 °C under N_2 gas until use. Fmoc amino acids were purchased from Novabiochem (San Diego, CA). Peptide synthesis resin and other chemicals for synthesis were purchased from Applied Biosystems (Foster City, CA). HPLC grade acetonitrile was purchased from VWR (Radnor, PA) and was filtered through a 0.22-mm

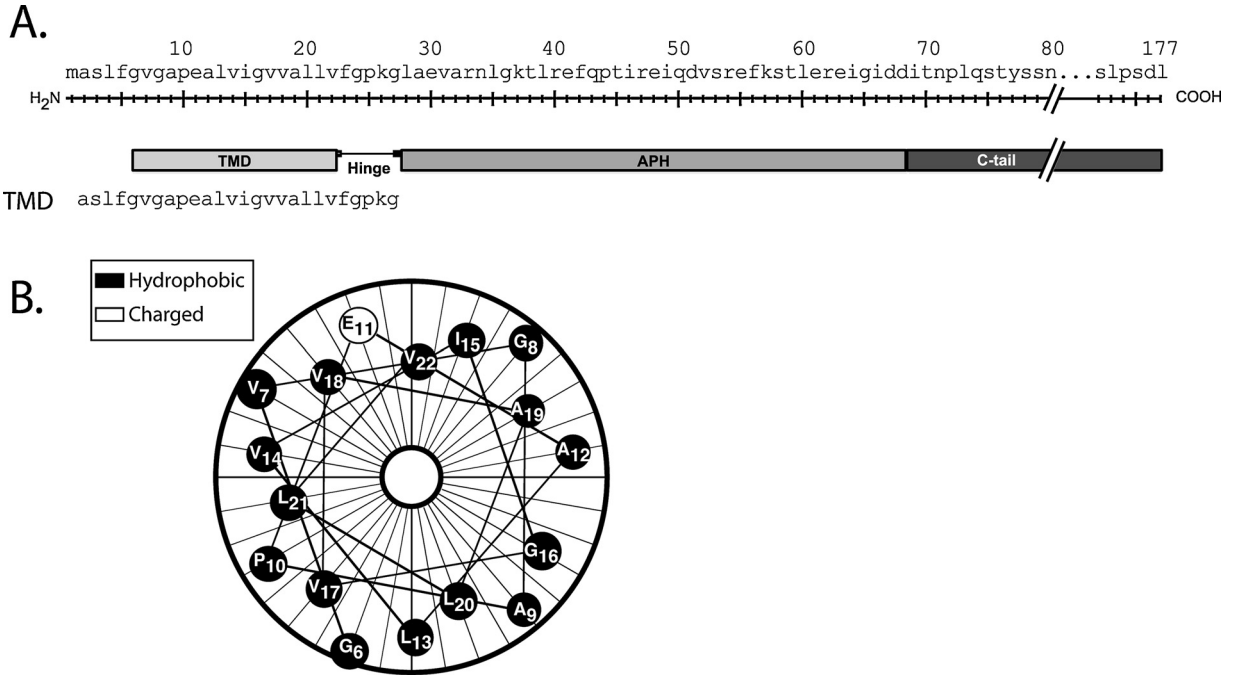

Fig. 1. Schematic of Hcf106 and peptide used in this study. Peptide Hcf106 TMD was synthesized on a CEM microwave peptide synthesizer (Matthews, NC) using Fmoc solid-phase peptide synthesis chemistry as described in the Materials and Methods. (A) A schematic of Hcf106 showing the different domains. The amino acid sequence of the peptide representing the TMD is indicated. (B) A helical wheel representation of the Hcf106 TMD peptide.