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Structural changes in a binary mixed phospholipid bilayer of DOPG and DOPS upon saposin C interaction at acidic pH utilizing ^{31}P and ^{2}H solid-state NMR spectroscopy

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Abstract

Saposin C (Sap C) is known to stimulate the catalytic activity of the lysosomal enzyme glucosylceramidase (GCase) that facilitates the hydrolysis of glucosylceramide to ceramide and glucose. Both Sap C and acidic phospholipids are required for full activity of GCase. In order to better understand this interaction, mixed bilayer samples prepared from dioleoylphosphatidylglycerol (DOPG) and dioleoylphosphatidylserine (DOPS) (5:3 ratio) and Sap C were investigated using ^{2}H and ^{31}P solid-state NMR spectroscopy at temperatures ranging from 25 to 50 °C at pH 4.7. The Sap C concentrations used to carry out these experiments were 0 mol%, 1 mol% and 3 mol% with respect to the phospholipids. The molecular order parameters (S_{CD}) were calculated from the dePaked ^{2}H solid-state NMR spectra of Distearoyl-d70-phosphatidylglycerol (DSPG-d70) incorporated with DOPG and DOPS binary mixed bilayers. The S_{CD} profiles indicate that the addition of Sap C to the negatively charged phospholipids is concentration dependent. S_{CD} profiles of 1 mol% of the Sap C protein show only a very slight decrease in the acyl chain order. However, the S_{CD} profiles of the 3 mol% of Sap C protein indicate that the interaction is predominantly increasing the disorder in the first half of the acyl chain near the head group (C1–C8) indicating that the amino and the carboxyl termini of Sap C are not inserting deep into the DOPG and DOPS mixed bilayers. The ^{31}P solid-state NMR spectra show that the chemical shift anisotropy (CSA) for both phospholipids decrease and the spectral broadening increases upon addition of Sap C to the mixed bilayers. The data indicate that Sap C interacts similarly with the head groups of both acidic phospholipids and that Sap C has no preference to DOPS over DOPG. Moreover, our solid-state NMR spectroscopic data agree with the structural model previously proposed in the literature [X. Qi, G.A. Grabowski, Differential membrane interactions of saposins A and C. Implication for the functional specificity, J. Biol. Chem. 276 (2001) 27010–27017] [1].

Keywords: Saposin C; Dioleoylphosphatidylglycerol; Dioleoylphosphatidylserine; Solid-state NMR spectroscopy; Molecular order parameter; Mixed bilayer

1. Introduction

Saposins are a family of a small 80 amino acid heat stable glycoproteins that are essential for the in vivo hydrolytic activity of several lysosomal enzymes in the catalytic pathway of glycosphingolipids [2,3]. Four members of the saposins family, A, B, C and D are proteolytically derived from a single precursor protein, named prosaposin [4,5]. Saposin C (Sap C) is known to stimulate the catalytic activity of the lysosomal enzyme Glucosylceramidase (GCase) and thereby to facilitate the hydrolysis of glucosylceramide to ceramide and glucose [6,7]. In addition, the presence of both Sap C and acidic phospholipids such as phosphatidylserine (PS) is required for

Abbreviations: Sap C, Saposin C; S_{CD}, Molecular Order Parameters; CSA, Chemical Shift Anisotropy; GCase, Glucosylceramidase; CL, Cardiolipin; DOPG, Dioleoylphosphatidylglycerol; DOPS, Dioleoylphosphatidylserine; PC, Phosphoatidyecholines; PG, Phosphatidylglycerol; PS, Phosphatidylserine; DSPG-d70, Distearoyl-d70-phosphatidylglycerol; DMPC, Dimyristylphosphatidylcholine; HCL, Hydrochloric Acid; TFE, 2,2,2 Triflouroethanol; PLL, Poly(L-lysine); NMR, Nuclear Magnetic Resonance; CP-MAS, Cross-Polarization Magic-Angle Spinning; HPLC, High Performance Liquid Chromatography; MALDI-TOF, Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry; MLVs, Multilamellar vesicles

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full activity of GCase [8–10]. Deficiency in either Sap C or GCase leads to different variant forms of Gaucher's disease [9–12]. Gaucher's disease is the most common genetic disease affecting Jewish people of Eastern European ancestry and is the most common lipid-storage disorder [13]. In addition, the disease course is quite variable, ranging from no outward symptoms to severe disability and death [13].

Under acidic pH conditions, Sap C is thought to destabilize the phospholipid membrane and facilitate the association of GCase with acidic phospholipid [12,14]. The binding of Sap C to phospholipid vesicles is a pH-controlled reversible process [14]. Since Sap C is a lysosomal protein and pH gradients occur in lysosomes in vivo, the degradation of lipids in the lysosome is proposed to be switched on and off by Sap C [14]. The three-dimensional structure of Sap C has been determined via solution NMR spectroscopy and Sap C was found to have five α-helices [14]. The negatively charged electrostatic surface of Sap C, resulting from its eleven Glu residues (See Fig. 1), needs to be partially neutralized to promote membrane binding [14]. In addition, the solution NMR structure of human Sap C in a detergent environment has been determined [15]. Using fluorescence emission spectroscopy and quenching analysis, a hypothetical model of membrane interactions with Sap C was proposed by Xiaoyang Qi and coworkers [1]. In this model, the amphipathic amino terminus of helix 1 and the carboxyl terminus of helix 5 of Sap C are inserted into the membrane and the middle region of Sap C is exposed to the aqueous phase. The positively charged Lys residues of Sap C, consolidated at 50% of the amino terminal half, are important in recognizing the negative surface of PS-containing membranes for initial binding of Sap C [1].

In addition to its role as GCase activator in the lysosome, Sap C participates in the fusion and destabilization of acidic phospholipids vesicles [12]. Membrane fusion is an important event in secretion, endocytosis, exocytosis, fertilization and intracellular transport [16]. Ying Wang and coworkers studied the mechanism of Sap C-induced membrane fusion using a particle size analyzer and fluorescence spectroscopy [17]. They proposed a "clip-on" model for Sap C-induced membrane fusion. This model proposes that helices 1 and 5 of two Sap C molecules are embedded into two different lysosomal phospholipid bilayer membranes and that membrane fusion is facilitated by the dimerization of Sap C (via helices 2–4) on opposing liposomal membranes [17]. Sap C is fusogenic, since it promotes the biological membrane fusion under acidic pH condition [6,18]. The fusogenic activity of Sap C is influenced by the composition of the membrane [6].

Solid-state NMR spectroscopy has been widely used to study the structure and dynamic of membrane–protein systems [19–24]. Solid-state NMR spectroscopy is an excellent technique for investigating lipid–protein interactions in membranes. Liposomes or phospholipid dispersions are commonly used to mimic and study biological membranes upon peptide insertion and interaction [20,25]. One aim of this study is to study the effect of anchoring of Sap C on the dynamics of the acyl chain of the phospholipid bilayers. ^{2}H solid-state NMR spectroscopy has been reported in the literature to study the effect of peptides altering the dynamic properties of phospholipid acyl chains [26,27]. Measuring the ^{2}H quadrupolar splittings and the disorder over the entire chain length of predeuterated phospholipids upon peptide binding provide a powerful tool to achieve this aim [28].

Recently, the interaction of the fusogenic peptide B18 in its amyloid-state with lipid membranes has been studied via solid-state NMR spectroscopy [29]. On the basis of ^{2}H and ^{31}P solid-state NMR spectroscopy results, it was possible to obtain a differentiated picture of the influence of the B18 peptide on different regions of the phospholipid bilayer. Moreover, ^{2}H and ^{31}P solid-state NMR spectroscopy has been used to study the interaction of Lantibiotic Nisin with mixed lipid bilayers [22]. Additionally, cytochrome *c* has been reported to effect phosphoatidyecholines (PC) more than Cardiolipin (CL) in the PC/CL bilayers using ^{31}P solid-state NMR spectroscopy [30]. In this paper, for the first time, ^{2}H and ^{31}P solid-state NMR spectroscopy has been used to investigate the structural changes in the lipid bilayer upon interaction of the fusogenic peptide Sap C with a PS-containing mixed binary lipid bilayer. Acidic phospholipids such as phosphatidylserine (PS) are required for the full activity of GCase [8,9]. Comparing the magnitude of interaction of Sap C with mixed acidic phospholipids is crucial to understanding the mechanism of Sap C interacting with membranes. Therefore, the second aim of this study is to determine whether Sap C interacts stronger with PS phospholipid than another acidic phospholipid such as phosphatidylglycerol (PG). PG is used in this study because it is a good candidate for studying the interaction of peptides and proteins with phospholipid bilayers [31–33]. Consequently, the binary mixed bilayers of the two acidic phospholipids were used to see if Sap C has a preference to one acidic phospholipid over another. In addition, the molecular miscibility of the two phospholipids (PG/PS) in the mixed binary lipid bilayers will be discussed [34].

2. Materials and methods

2.1. Materials

All synthetic phospholipids such as dioleoylphosphatidylglycerol (DOPG), dioleoyl-phosphatidylserine (DOPS) and distearoyl-d70-phosphatidylglycerol (DSPG-d70) were purchased from Avati Polar Lipids (Alabaster, AL). Sodium acetate (anhydrous) was purchased from (Fisher Scientific). Prior to use, the

1 10 20 30 40 50 60 70 80

SDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKMCSKLPKSLSEECQEVVDTYGSSILSLSILLEEVSPELVCSMLHLCSG

Fig. 1. The amino acid sequence of Sap C.