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StarD7 behaves as a fusogenic protein in model and cell membrane bilayers ☆

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ABSTRACT

StarD7 is a surface active protein, structurally related with the START lipid transport family. So, the present work was aimed at elucidating a potential mechanism of action for StarD7 that could be related to its interaction with a lipid–membrane interface. We applied an assay based on the fluorescence de-quenching of BD-HPC-labeled DMPC–DMPS 4:1 mol/mol SUVs (donor liposomes) induced by the dilution with non-labeled DMPC–DMPS 4:1 mol/mol LUVs (acceptor liposomes). Recombinant StarD7 accelerated the dilution of BD-HPC in a concentration-dependent manner. This result could have been explained by either a bilayer fusion or monomeric transport of the labeled lipid between donor and acceptor liposomes. Further experiments (fluorescence energy transfer between DPH-HPC/BD-HPC, liposome size distribution analysis by dynamic light scattering, and the multinuclear giant cell formation induced by recombinant StarD7) strongly indicated that bilayer fusion was the mechanism responsible for the StarD7-induced lipid dilution. The efficiency of lipid dilution was dependent on StarD7 electrostatic interactions with the lipid–water interface, as shown by the pH- and salt-induced modulation. Moreover, this process was favored by phosphatidylethanolamine which is known to stabilize non-lamellar phases considered as intermediary in the fusion process. Altogether these findings allow postulate StarD7 as a fusogenic protein.

1. Introduction

Membrane fusion is a ubiquitous event in cell biology, and indeed potentially all membranes can be merged under appropriate conditions. Fusion is involved in processes such as fertilization, cell differentiation (myoblasts and trophoblasts), transport of vesicles and protein-mediated virus entry to host cells. Many effectors are involved in the fusion process (e.g. intrinsic and extrinsic membrane proteins [1]; calcium, in exocytosis [2]; hormones, growth factors and antibodies, in receptor-mediated endocytosis [3]; pH, during the invasion of viruses [4]; etc.). While it is known that proteins and other factors are essential in controlling the membrane fusion process, lipids are the ultimate actors in this phenomenon. There are some lipids such as lysophosphatidylcholine, monoglycerides, phosphatidylethanolamine and phosphatidylserine that have been postulated as fusogenic in model membranes because they play a key role in membrane fusion [5]. However, it is unlikely that a particular lipid can be fully responsible for fusion in natural cell membranes.

The transport of lipids and proteins between organelles is also an essential process in the organization of different cellular compartments. Phospholipids are predominantly synthesized in the endoplasmic reticulum and subsequently transported to various destinations by vesicular transport through the fusion of vesicles to an acceptor compartment or can also be delivered to specific cellular organelles by monomeric exchange [6]. Several cytosolic proteins with specific lipid binding domains capable of accelerating lipid exchange in vitro have been identified. Based upon the homology in the domain responsible for interacting with the lipids, these proteins are grouped into six families called CRAL/TRIO, PITP, ORP, GLTP, SCP and START [7]. Crystallographic studies on the START domain of different members of this superfamily show that the three-dimensional organization of the domain forms a hydrophobic tunnel which is wide enough to accommodate the lipid molecule [6,8,9].

StarD7 is a member of the START domain protein family. Recombinant StarD7 protein is a surface active protein capable of interacting with lipid monolayers, particularly with negatively charged phospholipids such as phosphatidylserine and phosphatidylglycerol [10].

Abbreviations: DPH-HPC, 2-(3-(diphenylhexatrienyl) propanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine; BODIPY-FL-C5-HPC (also BD-HPC), 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine; DLS, dynamic light scattering; DMPC, dimiristoylphosphatidylcholine; DMPS, dimiristoylphosphatidylserine; DMPE, dimiristoylphosphatidylethanolamine; StarD7, StAR-related lipid transfer (START) domain containing 7; LUVs, large unilamellar vesicles; FRET, fluorescence energy transfer; MLVs, multilamellar vesicles; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; START, StAR-related lipid transfer; StAR, steroidogenic acute regulatory protein; SUVs, small unilamellar vesicles.

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A recent study demonstrated that the overexpression of StarD7 in a mouse hepatoma cell line induced the increase in the intracellular transport of exogenously incorporated phosphatidylcholine (PC) into the mitochondria. Although it was suggested that StarD7 facilitated the delivery of PC to mitochondria in non-vesicular form, the precise mechanism by which PC was trafficked remained to be elucidated [11]. Moreover, there is an increase in StarD7 expression and partial relocalization from the cytoplasm to the plasma membrane during syncytialisation, in vitro, of primary cytotrophoblasts from human placentae [12]. Syncytialisation is a differentiation process, which involves the intercellular fusion of trophoblast cells, concomitant with the translocation of phosphatidylserine from the inner to the outer leaflet of the trophoblast plasma membranes [13]. To interpret these results there might be two scenarios: a) StarD7 is involved in trophoblast differentiation through the transport of phosphatidylserine through the START domain [12] and/or b) StarD7 induces structural changes in the bilayer phase leading to membrane fusion. Based on its structural homology with other proteins of the START family, StarD7 might be postulated as a lipid carrier protein. However, an effect of StarD7 on trophoblast syncytialisation would support the protein has a fusogenic activity.

The present work was aimed at elucidating the mechanism of action of StarD7 derived from its interaction with a lipid–membrane interface.

2. Materials and methods

2.1. Materials

StarD7 (MW 34,697 Da), with a 95% purity as evidenced by SDS-PAGE, was overexpressed in *Escherichia coli* and purified as described previously [10]. The acyl chain labeled fluorescent probes (Fig. 1) DPH-HPC and BODIPY-FL-C5-HPC (BD-HPC) were purchased from Molecular Probes, Inc (Eugene, OR) and Avanti Polar lipids (Alabaster, AL), respectively. DMPC, DMPE and DMPS were purchased from Avanti Polar lipids (Alabaster, AL). Other chemicals were of analytical grade. Ultrapurified water was used all through the experiments.

2.2. Fluorescence measurements

Fluorescence spectra were recorded with a Fluoromax Spex-3 Jovin Yvon (Horiba, NJ, USA) spectrofluorimeter equipped with a thermostatized cell, a Xe arc lamp and a photomultiplier tube as signal detector, where light intensity was registered by a photon counting system. The excitation and emission slits were 2 nm wide. Emission spectra were recorded between 500 nm and 600 nm at 490 nm excitation wavelength.

2.3. Multilamellar vesicle (MLV) preparation

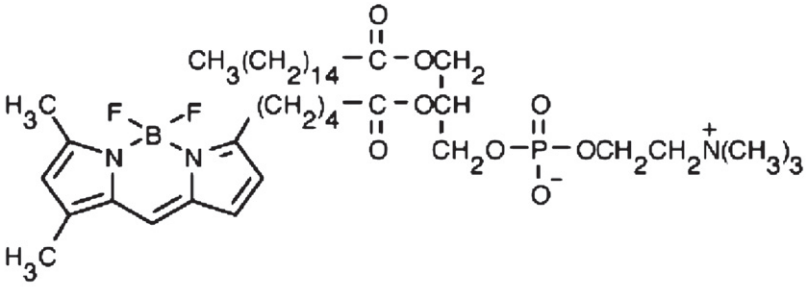
Lipids were dissolved in chloroform/methanol (2:1 v/v). The solvent was then evaporated under a stream on nitrogen with constant rotation of a test tube so as to deposit a uniform film over the bottom third of a tube and traces of solvent were removed under vacuum. The film was hydrated with 20 mM Tris–HCl, pH 7.8 by vortexing at a temperature above the gel to liquid-crystalline phase transition temperature of the lipids to obtain MLVs. The composition of "Donor" and "Acceptor" vesicles and their relative proportions used in the present work was as reported [14,15].

2.4. Donor liposomes

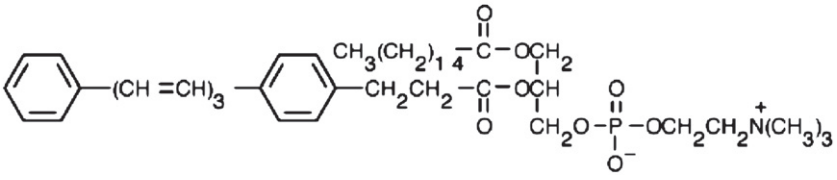
Small unilamellar vesicles (SUVs) were prepared essentially according to Pereira-Lachataignerais and co-workers [16] using a high intensity sonicator series Autotune, model 130 W with a 3 mm-diameter titanium probe and at a high frequency of 20 kHz. Ten mL of MLV dispersion containing 1:3.6 (mol/mol) mixture of BD-HPC (40 nM, final concentration) with phospholipids (DMPC–DMPS 4:1 mol/mol) was sonicated for 50 min (50 pulses with intervals of 15 s.) at 30 W in a 15 mL tube maintained on ice. Thus, labeled SUVs ($SUV_{BD\text{-}HPC/PC/PS}$) were obtained and used as donor liposomes. Non-labeled SUVs ($SUV_{PC/PS}$) were also prepared by applying the same procedure but in the absence of BD-HPC. Samples were centrifuged at 10,000 rpm for 2 min in order to release titanium particles and maintained covered with aluminum foil to prevent light incidence.

2.5. Acceptor liposomes

A MLVs' suspension containing 6 μM phospholipid concentration (DMPC:DMPS 4:1) was extruded across polycarbonate 100 nm pore size filters, according to MacDonald et al. [17] with a mini-extrusor



BODIPY-FL-C5-HPC



DPH-HPC

Fig. 1. Chemical structures of the fluorescence probes used.