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Surface activity and interaction of StarD7 with phospholipid monolayers☆

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Abstract

StarD7 protein forms stable Gibbs and Langmuir monolayers at the air–buffer interface showing marked surface activity. The latter is enhanced by penetration into phospholipid films at an initial surface pressure above the protein's own equilibrium adsorption surface pressure to a lipid-free interface. The protein–phospholipid stabilizing interactions at the interface depend on the lipid, with preference for phosphatidylserine, cholesterol, and phosphatidylglycerol, and the increases of lateral surface pressure generated are comparable to those of other membrane-active proteins. The surface activity of StarD7 is strong enough to thermodynamically drive and retain StarD7 at the lipid membrane interface where it may undergo lipid-dependent reorganization as indicated by changes of surface pressure and electrostatics.

Keywords: StarD7; Phospholipid monolayers

StAR-related lipid transfer domain superfamily is a rare case of adaptation of a protein fold with a conserved ligand-binding domain. In addition to participating in lipid transport, START domain containing proteins may target proteins to membranes [1,2] and/or perform certain catalytic functions [3]. It has been reported that human and mouse genomes each have 15 genes encoding START domains and phylogenetic analysis divides the family into six subfamilies [4]. The lipid ligands of these representatives START domain family have been identified only in some of them such as sterols (steroidogenic acute phase response protein) and phosphatidylcoline (PCTP) [1].

We have previously described the cloning and characterization of a new gene up-regulated in the choriocarcinoma JEG-3 cell line, denominated GTT1 (gestational trophoblastic tumor 1) [5]. Nucleotide sequence analysis of the cDNA and computer-assisted homology search of the deduced amino acid sequence showed approximately 25% identity and 49% similarity with human, bovine or mouse PCTP. In addition, GTT1 shares a conserved extended central region—from amino acids 66–250—with the START domain proteins proposed to bind lipids and interact with membranes. For this reason GTT1 was renamed StarD7 (START domain containing 7) herein called StarD7.

A recent report includes StarD7 into the PCTP subfamily together with PCTP, StarD10, and Goodpasture antigen-binding protein [4]. Even though StarD7 shares similarity or identity in 20 of the 28 residues in the hydrophobic cavity with PCTP the characteristics of the lipid molecule in StarD7 have not been identified. The crystal structure of PCTP suggests that lipid entering or exiting would require a major conformational change unfolding the C-terminal α-helix lid [6]. The open

☆ *Abbreviations:* START, StAR-related lipid transfer; StAR, steroidogenic acute regulatory protein; PCTP, phosphatidylcholine transfer protein; GTT1/StarD7, gestational trophoblastic tumor 1/START domain containing 7; PC, phosphatidylcholine; IPTG, isopropyl β-D-thiogalactopyranoside; PG, phosphatidylglycerol; dpPC, dipalmitoylphosphatidylcholine; dlPC, dilaurylphosphatidylcholine; PS, phosphatidylserine; Cho, cholesterol; Sphm, sphingomyelin.

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structure of PCTP is taken to represent the membrane-associated form, where the lipid-exchange loop acts as a membrane anchor. In order to know if an interaction with membrane may occur with StarD7 protein, we studied the surface activity of StarD7 and its penetration into interfaces of different lipids, and disclosed surface-active properties that may enable this protein to interact with defined phospholipids.

Materials and methods

Materials. Restriction endonucleases were obtained from US Biochemical, Cleveland, OH.

All other chemicals were of the highest purity available and were purchased from Sigma Chemical.

Overexpression and purification of the StarD7 protein. Total RNA of JEG-3 choriocarcinoma cell line was extracted using the acid guanidinium thiocyanate–phenol–chloroform extraction method described by Chomczynski and Sacchi [7]. Single-stranded cDNA was synthesized with StarD7 antisense primer (5′-TATCCCAAAGCCTG TCAAGCATACTCAATCCG-3′) in 20 μl reaction. One microgram of total RNA was incubated with 1.5 μM of the above primer, 1 U/μl RNasin ribonuclease inhibitor (Promega), and single-strength M-MLV reverse transcriptase buffer (50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 10 mM DTT) at 65 °C for 15 min. After the addition of 1 mM dNTPs and 200 U Moloney Murine Leukemia Virus-Reverse Transcriptase (MMLV-RT, Promega), the reaction mixture was incubated at 42 °C for 1 h. PCR amplification was performed with one set of PCR primers, including the sense (5′-GCCTCTGgtaccATGG CGGCGTTA-3′) and antisense (5′-TATCCCAAAGggtaccAAGCAT ACTCAATCCG-3′) primers. The amplification protocol included: 1 cycle at 94 °C for 2 min; 20 cycles at 94 °C for 30 s, 56 °C for 1 min, and 68 °C for 3 min; 1 cycle at 68 °C for 10 min; and 1 cycle at 4 °C for 10 min. The primers were designed to contain a *Kpn*I site (small letters) for cloning purpose. The amplified fragment was cloned into the pGEMTeasy (Promega). Subsequently, the recombinant clone was cut with *Kpn*I restriction enzyme and cloned into the *Kpn*I site of pRSET expression vector (Invitrogen), which carries NH_2-terminal His-tag/EK configuration. The plasmid containing the desired sequence was selected and checked by sequencing. pRSET-StarD7 construct was transformed into the BL21(DE_3)-competent *Escherichia coli* cells. The optimal expression conditions of recombinant StarD7 were 0.1 mM IPTG induction for 4 h. All subsequent manipulations were performed at 4 °C. Bacteria were harvested by centrifugation at 5000*g* for 25 min. The pellet was washed once with 10 mM Tris–HCl, pH 7.4, 500 mM NaCl, 10% glycerol, 5 mM DTT, 0.1% NP-40, and protease inhibitor, and then resuspended in the same buffer. The resuspended cells were lysed by sonication. The homogenate was centrifuged at 10,000*g* for 30 min. The supernatant was collected and used to purify the StarD7 protein by Ni^{+2}-column chromatography (Novagen). The protein was eluted with a linear gradient of 10–500 mM imidazole, 100 mM KCl, and 20 mM Tris–HCl, pH 7.8, in the presence of protease inhibitors. The purity of the protein was estimated by SDS–PAGE, being more than 95% (data not shown). Purified protein was stored in 20% glycerol (v/v) at −20 °C.

Lipid monolayers. Protein films formed by adsorption or spreading and lipid monolayers spread from chloroform:methanol (2:1) were prepared in a specially designed apparatus, with a multiple compartment Teflon trough and compression barriers previously described [8]. Isothermal variation of the surface pressure and surface potential as a function of the mean molecular area under compression of Langmuir monolayers were determined automatically in one compartment (90 cm^2 of surface area and 76 ml subphase buffer 20 mM Tris–HCl, pH 8). Surface pressure was measured with a platinized Pt Wilhelmy plate transducer and the surface potential with a high measured impedance millivoltmeter through an air-ionizing ^{241}Am plate and a calomel subphase reference electrode pair. The increase of surface pressure at constant area due to formation of pure protein Gibbs monolayers, and to protein penetration pre-formed lipid monolayers, was measured in a smaller compartment of the trough (18 cm^2 of surface area and 17 ml of subphase buffer). Absence of surface active impurities in the aqueous solution was routinely controlled as reported before [8].

Results and discussion

Surface activity of StarD7

StarD7 forms stable Gibbs monolayers by adsorption at the air–buffer interface, after injecting the protein into the subphase as indicated by the increase of surface pressure at constant area as a function of time. The equilibrium adsorption process and its kinetics, as measured by the maximum increase of surface pressure (18 mN/m in about 5 min), are dependent on the subphase concentration of protein up to about 100 nM; no further increases are obtained at higher concentration, while at 50 nM protein in the subphase the same maximum equilibrium pressure is attained after a longer time (Fig. 1).

The maximum surface pressure attained by adsorption remained constant, indicating that a stable monolayer was formed at the interface and the adsorbed film could be compressed and decompressed reversibly by reducing or expanding, respectively, the available area further emphasizing the film stability. Also, this indicated that the protein film could acquire different intermolecular organizations depending on packing (see below), and that the Gibbs protein monolayer performed by adsorption corresponded to an intermediate one. This behavior is comparable to that described for extrinsic and intrinsic membrane proteins, membrane-

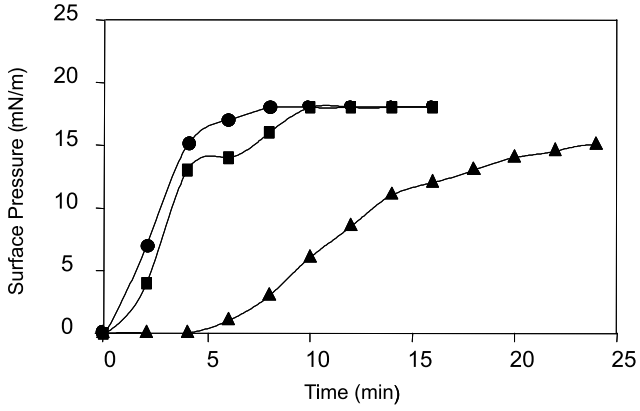

Fig. 1. Adsorption of StarD7 at the air-interface by injecting different protein concentrations into the subphase: 50 nM (triangle); 100 nM (square); and 200 nM (circle).

active and amphitropic proteins, and surface-active polypeptides [9–11].

StarD7 also spreads directly from aqueous solution as stable Langmuir monolayers. The high surface stability of the Langmuir protein film is reflected in the fact that surface pressure- and surface potential-mean molecular area isotherms are reproducible, with no desorption of interfacial protein, after successive expansion and recompression cycles. This also indicated that the same intermolecular organization can be adopted reversibly on compression or expansion, depending on the lateral surface pressure and on the initial packing state of the film. The dependence on the initial conditions is reflected by the hysteresis observed for the compression–expansion isotherms (Fig. 2) that is common for lipids and membrane-active proteins [10]. In the case of StarD7 the energy stored by the system during one full compression–expansion cycle, representing the cost of storing the amount of information required for the acquisition of the interfacial molecular organization at the different surface pressures (and according to the direction of its variation), corresponds to the rather high energy gap of about 36.9 kcal/mol. For other amphitropic proteins this value was reported to be of similar magnitude [9,12].

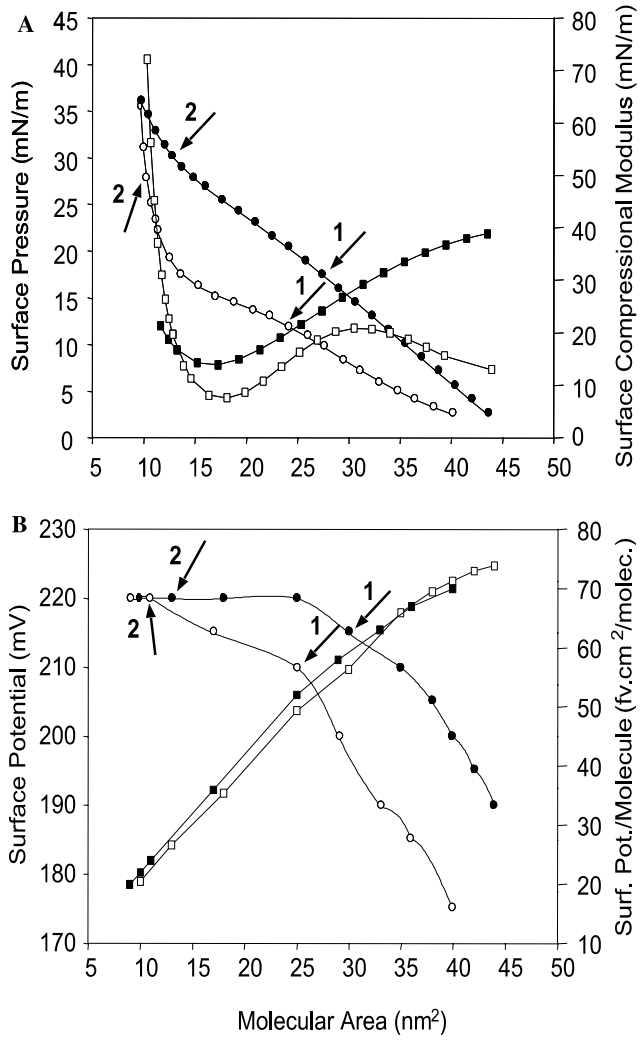

Fig. 2. (A) Compression (black circle) and decompression (white circle) isotherms with the corresponding surface compression modulus (black and white squares) of StarD7 Langmuir monolayer. (B) Surface potential of compression (black circle) and decompression (white circle) isotherms and the surface potential/molecule (black and white squares), respectively. Arrows indicate reversible rearrangement of the interfacial organization of StarD7. The arrows labeled 1 and 2 indicate the first and second inflexion points, respectively.

The collapse pressure of the StarD7 Langmuir film is also high, again revealing the marked surface activity and stability to collapse, being about 36 mN/m (within 2 mN/m). The collapse occurs in a diffuse manner at a cross-sectional mean molecular area between 9 and 10 nm^2 and a surface potential of about 220 mV (Fig. 2). The minimum mean molecular area attained just before collapse would be equivalent to that of a bundle formed by 5–6 α-helical chains perpendicular to the interface. On the basis of the surface molecular density at 18 mN/m (the maximum equilibrium pressure obtained by adsorption as a Gibbs monolayer) the overall free energy of the protein adsorption from the subphase, as a monomolecular layer, is −6.7 kcal/mol, a value comparable to that of other amphitropic proteins, reflecting its high tendency to interfacial adsorption [9–12].

A reversible rearrangement of the StarD7 interfacial organization occurs under compression between 15 and 20 mN/m and molecular packing areas of 30–25 nm^2 (centered at 18 mN/m and 26.6 nm^2); under the expansion process the change of organization is shifted to a lower surface pressure and smaller areas (centered at 12 mN/m and 23.4 nm^2). The protein rearrangement is clearly indicated by the variation of the surface compressional modulus of area compressibility (Fig. 2A) indicating the beginning and ending of reorganization between packing states of quite different surface elasticity, all belonging to a fairly liquid state [13]. A second inflexion point revealed by the surface compressional modulus is centered under film compression at a surface pressure of 30 mN/m (12.8 nm^2) and at 25 mN/m (10.7 nm^2) under expansion. This indicates that, above 20 mN/m, a more liquid-condensed state is obtained, with much less interfacial elasticity after the protein has been closely packed (as indicated by the higher values of the surface compressional modulus during expansion at molecular areas below about 12.5 nm^2). Hysteresis was also observed for the surface potential, depending on whether the protein film was compressed or expanded. Again, the change of protein interfacial organization is indicated by the variation of the slope of the curve representing the change of the surface potential per unit of molecular surface density [14], directly proportional to the protein resultant dipole moment perpendicular to the interface with the molecular packing area (bearing a positive end pointing away from the hydrophilic region

and into the hydrophobic air side) (Fig. 2B).This independent change occurs over the same packing area range at which the inflexion point is observed in the surface compressional modulus.

Association of StarD7 to lipid monolayers

Apart from its own surface activity and marked stabilization by interfacial adsorption to a lipid-free surface, StarD7 injected into the subphase solution readily penetrated spread lipid monolayers at initial surface pressures above the maximum equilibrium surface pressure attained in the Gibbs monolayer formed by the protein alone (18 mN/m). This clearly indicated the spontaneous thermodynamic tendency of StarD7 to interact with, and acquire further surface stability through association to, the lipids organized as a monolayer at the interface. The maximum surface pressure reached was different depending on the lipid in the monolayer showing the protein selectivity for preferential interactions. At an initial film pressure of 15–18 mN/m relatively high interfacial stabilization is found with PG, dlPC, PS, and Cho, intermediate for dpPC, and relatively lower for Sphm (Fig. 3).

For a given subphase concentration of StarD7 the well-known [15] inversely dependent protein interfacial penetration with the increase of the initial surface pressure of the lipid film was observed. Extrapolation of the line showing the variation of the surface pressure increase induced by the protein as a function of the initial surface pressure of the lipid film to the abscissa corresponds to the cut-off pressure point (Fig. 4). This represents the maximum initial surface pressure at which the protein can penetrate a lipid film and is related to the establishment of more or less favorable lipid–protein interactions. The cut-off pressure for Cho and the acidic phospholipids PG and PS reached values above 30–40 mN/m; meaning that the protein favorably penetrates those films and continues to do so until bringing the interface close to its own collapse pressure point. For the zwitterionic phosphatidylcholine the cut-off pressure occurs at 24 mN/m, this value corresponds to the equilibrium adsorption pressure of the pure protein or to the region at which the protein undergoes surface pressure-induced interfacial reorganization.

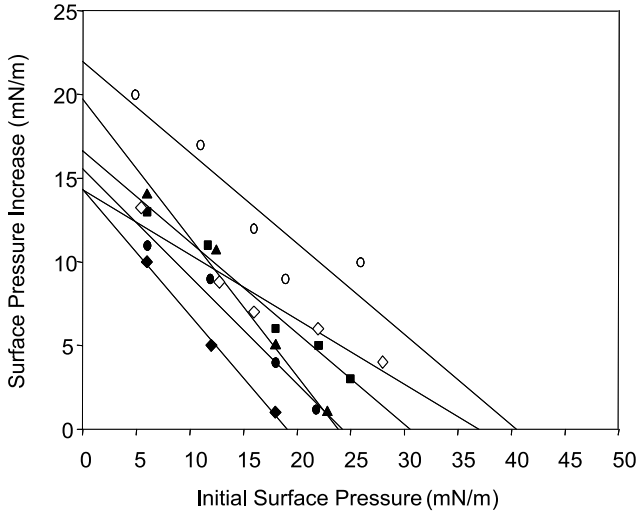

Fig. 4. Dependence of the surface pressure increase with the initial surface pressure (cut-off plot) induced by injection of 100 nM of StarD7 into the subphase of spread lipid monolayers. Phosphatidylserine (white circle); cholesterol (white rhombus); phosphatidylglycerol (black square); dilaurylphosphatidylcholine (black triangle); dipalmitoylphosphatidylcholine (black circle); and sphingomyelin (black rhombus).

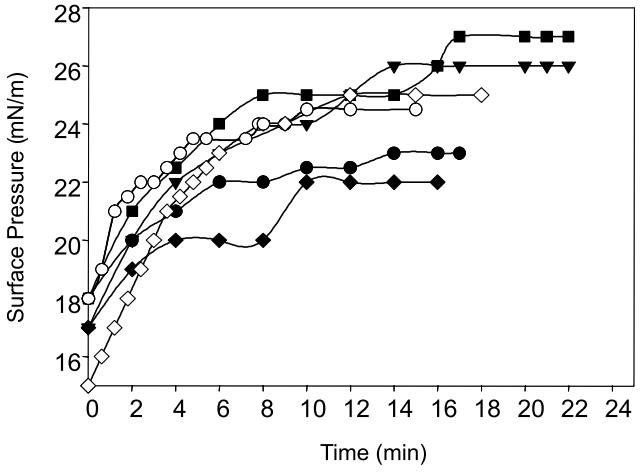

Fig. 3. Penetration of StarD7 to different lipids monolayers. Phosphatidylserine (white circle), phosphatidylglycerol (black square), dilaurylphosphatidylcholine (black triangle), cholesterol (white rhombus), dipalmitoylphosphatidylcholine (black circle), and sphingomyelin (black rhombus). The initial surface pressure was set at 15–18 mN/m before subphase injection of protein.

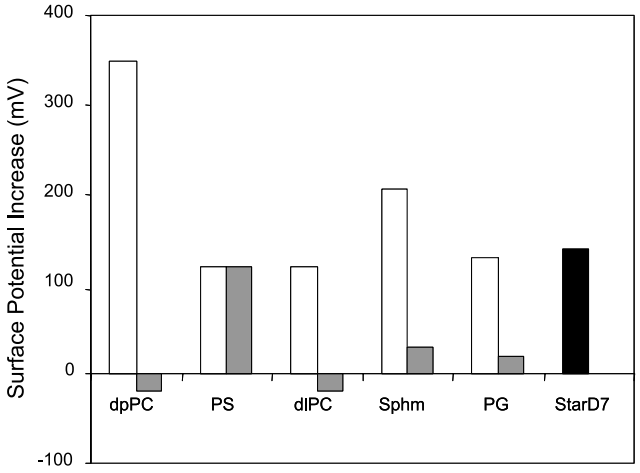

Fig. 5. Surface potential increase induced by penetration of StarD7 into different lipid monolayers at high (18 mN/m, grey bars) or low (6 mN/m, white bars) initial surface pressure. Interfacial adsorption to a lipid-free surface of StarD7 (black bars). PG, phosphatidylglycerol; dpPC, dipalmitoylphosphatidylcholine; dlPC, dilaurylphosphatidylcholine; PS, phosphatidylserine; and Sphm, sphingomyelin.

The change of surface potential caused in the lipid monolayer by the protein penetration at relatively low initial surface pressure (6 mN/m) shows considerable increases in positive values (Fig. 5). The surface potential is directly proportional to the resultant dipole moment perpendicular to the interface [14]. These results indicate that the change of overall mean dipole moment resulting from the lipid–protein interactions in the protein penetrated film is in the same direction as that of the pure protein film, pointing with the positive (air) end toward the methyl end of the lipid hydrocarbon chains [8]. At relatively high surface pressures (18 mN/m) the mean resultant dipole moment is reduced (with all lipids except for PS), as compared to that at low surface pressure. This could be due to a change of interfacial location, to molecular organization of the protein (see Fig. 2), to a lesser amount of protein penetrating the film at high compared to low surface pressures (see Fig. 4) or to a combination of effects. These factors cannot be separately disclosed by the measurements done so far. This type of effect has also been reported for other amphitropic proteins [10]. Clearly, the type of lipid polar head group has a marked influence on the possibilities for interfacial protein penetration which, in turn, affects the protein interfacial stability and/or molecular organization as indicated by the variations of surface electrostatics.

Taken together these results indicate that the surface activity of StarD7 is strong enough to thermodynamically drive and retain the protein at the lipid membrane interface where it may undergo lipid-dependent reorganization as indicated by changes of surface pressure and electrostatics. In addition, these data support our proposal that StarD7 may play an important role in the phospholipid-mediated signaling of trophoblastic tumor cellular events [5].

Acknowledgments

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