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Short communication

Self-assembly of nanoscale particles with biosurfactants and membrane scaffold proteins

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ABSTRACT

Nanodiscs are membrane mimetics which may be used as tools for biochemical and biophysical studies of a variety of membrane proteins. These nanoscale structures are composed of a phospholipid bilayer held together by an amphipathic membrane scaffold protein (MSP). In the past, nanodiscs were successfully assembled with membrane scaffold protein 1D1 and 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine with a homogeneous diameter of ~10 nm. In this study, the formation of nanoscale particles from MSP1D1 and rhamnolipid biosurfactants is investigated. Different protein to lipid ratios of 1:80, 1:90 and 1:100 were used for the assembly reaction, which were consecutively separated, purified and analyzed by size-exclusion chromatography (SEC) and dynamic light scattering (DLS). Size distributions were measured to determine homogeneity and confirm size dimensions. In this study, first evidence is presented on the formation of nanoscale particles with rhamnolipid biosurfactants and membrane scaffold proteins.

1. Introduction

Phospholipids are a major component of all native cell membranes and have been used in the past for the assembly of discoidal phospholipid bilayer nanoparticles, so called nanodiscs [1]. Nanodiscs are composed of a phospholipid bilayer held together by an amphipathic membrane scaffold protein (MSP) which is based on the apolipoprotein A-I sequence but without the globular N-terminal domain [2]. Nanodiscs represent a class of membrane mimetics and provide an impressive tool for biochemical and biophysical studies of a variety of membrane proteins [1] including G-protein coupled receptors [3], ion channels [4], or pores and toxins [5,6].

It is known that microorganisms like bacteria, yeasts and fungi produce biosurfactants which are amphiphilic surface-active substances [7]. Extensive research has been performed on rhamnolipids (RLs), which belong to the group of glycolipid biosurfactants [8]. Rhamnolipids are composed of a glycon part comprising one (mono-RLs) or two (di-RLs) rhamnose moieties and an aglycon part which consists most commonly of one or two saturated β-hydroxyfatty acid chains which are linked through a α-1,2-glycosidic linkage [9,10]. Due to the amphiphilic character, structure and relative size of polar head groups versus hydrophobic tail, rhamnolipids show similarities to phospholipids. Phospholipids, particularly glycerophospholipids consist of a glycerol backbone at the *sn-3* position with acyl moieties at the *sn*-1 and *sn*-2 positions, respectively [11].

The formation of nanodiscs is initiated by the removal of detergent from a starting mixture containing a defined ratio of MSPs, lipids and detergent [12]. Three parameters are relevant for a successful assembly of nanodiscs: 1) the lipid to protein stoichiometry, 2) the choice of detergent and 3) the assembly temperature [13]. Nanodiscs have been successfully assembled with various synthetic phospholipids namely 1,2-Dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) [14], 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) [15] or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine POPC [16] and lipid mixtures from natural sources, such as *E. coli* polar lipids [17] or *E. coli* total lipids [18].

With this knowledge, the aim of this study was to examine the potential self-assembly process of purified mono-rhamnolipids and di-rhamnolipids in the presence of the membrane scaffold protein 1D1 to nanoscale structures.

2. Material and methods

2.1. Materials

Chemicals used were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany) and Bio-Rad Laboratories, Inc. (Hercules, USA). The membrane scaffold protein 1D1, as lyophilized powder, was

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purchased from Sigma-Aldrich (Taufkirchen, Germany). The expression plasmid pMSP1D1 was a gift from Stephen Sligar, University of Illinois, Urbana, USA (Addgene plasmid # 20061) [19]. The synthetic phospholipid DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids (Alabaster, USA). The mono- and di-rhamnolipids are from own production at the department of Bioprocess Engineering at the University of Hohenheim, Germany as described in [20].

2.2. Methods

2.2.1. Preparation of the disc-samples

2.2.1.1. Reconstitution mixture with rhamnolipids. For preparing reconstitution mixtures for nanoscale particles with rhamnolipids, the volumes of MSP1D1, mono-rhamnolipid (RL1) and di-rhamnolipid (RL3) respectively, were calculated as described in Ritchie et al. [21] because of the similarities of phospholipids and rhamnolipids. For preparing a 100 mM stock of RL3, the granules were incubated for at least 30 min at 60 °C in a drying cabinet (UF110; Memmert GmbH + Co.KG; Schwabach, Germany). Then the desired amount of granulate was weighed and dissolved in 200 mM sodium cholate solution to reach a concentration of 100 mM. In order to obtain a 100 mM stock of RL1, the desired amount of RL1 was weighed and dissolved in 200 mM sodium cholate solution. To ensure complete resuspension, rhamnolipids were sonicated in an ultrasonic bath for 10 min at a frequency of 37, 50 % power and 30 °C under degassing (Elmasonic P; Elma Schmidbauer GmbH, Singen, Germany). The respective volume of protein was added to the sodium cholate solubilized RL1 or RL3 in glass vial with a PTFE-lined screw cap. The mixture was incubated in a sand bath placed on a hot plate for 1 h at circa 130 °C which was monitored with a thermometer.

2.2.1.2. Reconstitution mixtures with phospholipids. For preparing reconstitution mixtures for nanodiscs, the volumes of MSP1D1 and DPPC stock solution were calculated as described in Bayburt et al. [2], to yield an optimal molar ratio of 1:90 MSP1D1 to lipid [19]. Phospholipid stocks are prepared in chloroform at 100 mM and stored at −20 °C into a disposable glass culture tube with PTFE-lined screw caps.

2.2.2. Nanodiscs assembly and nanoscale particles with rhamnolipids assembly

The self-assembly of nanodiscs is initiated by sodium cholate removal by dialysis as described in Bayburt et al. [2]. With this knowledge the mixture of MSP1D1, rhamnolipids and cholate was treated equally because of the structural similarities and surfactant properties of phospholipids and rhamnolipids. The dialysis was performed with dialysis cassettes (Slide-A-Lyzer dialysis kit; Thermo Fischer Scientific; Waltham, USA). Briefly, the cassette was hydrated for 2 min in dialysis buffer containing 40 mM Tris/HCl and 100 mM NaCl, pH 8.0 [22] and was removed from the buffer for sample injection. Thereby the volume of buffer was approximately 1000 times of the sample volume. The lipid protein mixture was filled into a cassette syringe port with a hypodermic needle and the cassette was placed in dialysis buffer. Dialysis was conducted for 16 h at ambient temperature followed by buffer exchange and subsequent incubation for 16 h at 4 °C. Then the assembled nanodiscs and nanoscale particles with rhamnolipids were withdrawn into the syringe. The samples were filtrated using 0.20 µm cellulose acetate filter (Chromafil CA-20/15-S; Macherey-Nagel; Hoerdt, France) and stored at 4 °C.

2.2.3. Analytical procedure

2.2.3.1. Separation, purification and fractionation by size. Size exclusion chromatography (SEC) is applied for the final purification and analysis of the assembled nanodiscs and nanoscale particles with rhamnolipids. Thereby, the nanoparticles of both approaches were separated by size using a chromatography system (NGC chromatography system Quest; Bio-Rad Laboratories, Inc.;Hercules, USA) on a calibrated size exclusion chromatography column (Superdex 200 Increase 10/300 GL; GE Healthcare; Chicago, United States of America). Data were processed and analyzed using the chromatography system software (Chromlab™ 4.0 software; Bio-Rad Laboratories, Inc.; Hercules, United States of America). The elution profiles were monitored at 280 nm and relevant discs fractions were pooled and used for further analysis.

2.2.3.2. Particle size distributions and homogeneity. Particle size distribution and homogeneity of the generated nanodiscs and nanoscale particles with rhamnolipids were measured by dynamic light scattering (DLS) with a nanoparticle size analyzer (Zetasizer Nano ZS particle size analyzer; Malvern Instruments Ltd; Malvern, UK) equipped with a He-Ne laser as a light source (wave-length: 633 nm; detecting backscatter at: 173 °) at 25 °C. Samples were equilibrated to room temperature for at least 30 min, filtered using 0.20 µm cellulose acetate filter (Chromafil CA-20/15-S; Macherey-Nagel; Hoerdt, France) and were transferred into 4 mL disposable PS cuvette. DLS results are presented as averages of three independent measurements. The Data were analyzed by using the nanoparticle size analyzer software (Zetasizer software 7.04; Malvern Instruments Ltd; Malvern, UK) to yield number-based size distribution of the samples.

3. Results and discussion

Di-rhamnolipid (Rha-Rha-C_{10}-C_{10}) was mixed with lipid to MSP1D1 ratios of 1:80, 1:90 and 1:100 into nanoscale particles with the detergent-dialysis method as described by Bayburt et al. [2]. The assembly mixture was then separated by size exclusion chromatography (Fig. 1) using a calibrated column, and the hydrodynamic diameter and particularity was determined by dynamic light scattering (DLS).

Employing a protein to lipid ratio of 1:80, the elution profile indicated a shouldered peak at a retention volume at 14.2 mL (Fig. 1). Using the calculation proposed in reference [23] lead to a Stokes diameter of the particles in these approach of 7.8 nm. This mean diameter was confirmed with the DLS measurements to be 7.0 nm ± 1.4 nm, with a particle polydispersity index (PDI) of 0.433 (Table 1). A comparable result was obtained with a protein to lipid ratio of 1:90. When applying a MSP1D1 to rhamnolipid ratio of 1:100, it was visible that the main (right) peak shifted towards a higher elution volume of 15.1 mL, thereby achieving a separation from the smaller shoulder (left) peak at 13.8 mL (Fig. 1). The calculated diameter of the shoulder (left) peak was 8.3 nm, whereas the DLS measurements differed with a mean diameter of 5.0 nm ± 0.9 nm and a PDI of 0.655, indicating a non-homogeneous and much broader particle size distribution (Table 1). The main (right) peak at 15.1 mL corresponded to a Stokes diameter of 6.6 nm. This result was confirmed with DLS measurements of 6.0 nm ± 1.2 nm as the mean particle size in the sample. Moreover a narrower size distribution in the sample was indicated by a PDI of 0.452. When comparing to the control assembly reaction with pure MSP1D1, the (right) peak elutes at a similar volume of approx. 15 mL. This was furthermore performed using mono-rhamnolipid (Rha-C_{10}-C_{10}) and MSP1D1 accordingly, however, no conclusive results could be obtained.

In Fig. 1 the peak using a MSP:RL ratio of 1:80 and 1:90 at elution volumes of 14.2 mL and 14.1 mL and diameters of 7.8 nm and 7.9 nm, respectively, indicates the formation of particles. As no particles of this size could be detected in the controls, the formation of rhamnolipid/MSP nanoparticles suggests itself. Referring to the size of native MSP1D1 in the control reactions, lower elution volumes indicate a larger size upon assembly with di-rhamnolipid. This is furthermore confirmed by the assembly with increasing amounts of di-rhamnolipid (1:100, Fig. 1), which results in a shift towards higher elution volumes (right peak) and therefore smaller particles. This is likely due to the fact that self-assembly of micellar structures occurs at higher concentrations