Experimental report

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Proposal: 8-03-872 **Council:** 4/2016

Title: Interparticle lipid transfer in polymer-stabilised native nanodiscs

Research area: Biology

This proposal is a new proposal

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Samples: 1,2-dimyristoyl-sn-glycero-3-phosphocholine
d54-1,2-dimyristoyl-sn-glycero-3-phosphocholine
styrene/maleic acid

Instrument	Requested days	Allocated days	From	To
D22	3	2	08/07/2016	10/07/2016

Abstract:

Amphiphilic copolymers composed of styrene and maleic acid (SMA) are newly developed tools with the ability to form lipid-polymer complexes when mixed with biological membranes. These complexes, termed native nanodiscs or SMALPs, are discoidal nanoparticles capable of solubilising and stabilising both lipids and integral membrane proteins in solution without the aid of conventional detergents. Although the structural features of these particles have been partially unveiled, the kinetics governing trans-SMALP lipid exchange remain unknown. This is of utmost importance to fully exploit this new technology and understand the kinetics and, thus, the mechanism of (i) SMA-mediated lipid extraction from vesicles into SMALPs, (ii) the inter-SMALP lipid transfer and (iii) the reincorporation of lipids from nanodiscs into vesicular membranes. We will produce SMA-stabilised nanodiscs composed of hydrogenated SMA and either hydrogenated (H-) or deuterated (D-) phospholipid. We propose the use of time-resolved small-angle neutron scattering to investigate the interbilayer exchange kinetics among D-SMALPs and H-SMALPs in solution at various temperatures.

Fast lipid transfer among polymer-bounded nanodiscs as monitored by time-resolved small angle neutron scattering*

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Introduction

Several styrene/maleic acid (SMA) copolymers solubilise membrane proteins and surrounding lipids to form SMA/lipid particles (SMALPs) without requiring conventional detergents[1,2]. SMALPs are disc-shaped nanoparticles with typical diameters of 10–25 nm[3,4] that are made up of a lipid-bilayer patch bounded by a polymer belt[5,6]. Thus, their colloidal morphology is similar to that of lipid nanodiscs surrounded by amphipathic membrane scaffold proteins (MSPs)[7]. SMALPs are attracting great attention as a new membrane mimic because they can solubilise proteins from artificial[1,8] or natural[9] membranes while retaining a native-like environment in the form of a nanosized lipid bilayer. Owing to their small size, SMALPs are well suited for optical-spectroscopic[3,4,10,11] and chromatographic[12] techniques.

Nothing is known about the kinetics and the underlying mechanism(s) of lipid exchange, although such quantitative and mechanistic insights are essential if one is to fully exploit the potential of the SMALP technology for investigating biological membrane components *in vitro*. Here, we quantify the kinetics of lipid transfer among SMALPs formed from the zwitterionic phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and an SMA copolymer having a 3:1 styrene/maleic acid molar ratio (SMA(3:1)). Our results demonstrate that SMALPs are highly dynamic rather than kinetically trapped structures that rapidly exchange lipids, which has important implications for exploiting "native nanodiscs" in membrane-protein studies.

Experimental

Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (h-DMPC) was obtained from Lipoid (Ludwigshafen, Germany), acyl-chain deuterated 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phosphocholine (d-DMPC) from Cortecnet (Paris, France). SMA(3:1) copolymer solution (trade name XIRAN SL25010 S25) was a kind gift from Polyscope (Geleen, Netherlands). D_2O was from Deutero (Kastellaun, Germany), NaCl from VWR (Darmstadt, Germany), and tris(hydroxymethyl)aminomethane (Tris) from Carl Roth (Karlsruhe, Germany). All chemicals were obtained in the highest purity available.

TR-SANS

h-SMALPs and d-SMALPs were produced by solubilising MLVs made from either h- or d-DMPC with SMA(3:1) at 30 °C for 16 h to yield final concentrations of 20 mM lipid and 3.3 mM SMA(3:1). Both d- and h-SMALPs were formed in buffer (50 mM Tris, 200 mM NaCl, pH 7.4) containing either 77.5% or 0% (*v*/*v*) D_2O. Complete solubilisation was confirmed by DLS as described above.

TR-SANS measurements were performed on the D22 beam line at the Institut Laue–Langevin (Grenoble, France). The beam line is fed by a cold neutron source that emits monochromatic neutrons at $\lambda = (6.0 \pm 0.6)$ Å and is equipped with an SFM-300 stopped-flow apparatus (Biologic, Seyssinet-Pariset, France) with a 200-µL quartz glass cell having a pathlength of 1 mm. The instrument was set up for rectangular collimation of 40 mm x 55 mm at a sample/detector distance of 5.6 m and a sample aperture of 7 mm x 10 mm. The drive syringes, tubes, and quartz cuvette were temperature-controlled, and samples were equilibrated for at least 5 min prior to each measurement. The D_2O concentration in the buffer was adjusted to 42.8% (*v*/*v*) to match the SLD contrast of SMALPs harbouring equal amounts of h- and d-DMPC. This condition was experimentally determined from static SANS measurement of h-SMALPs and d-SMALPs in 77.5% and 0% (*v*/*v*) D_2O. 450-µL aliquots of d- and h-SMALPs in 42.3% (*v*/*v*) D_2O were mixed at a total speed of 5 mL/s at 11.1, 15.1, 22.1, 27.5 and 32.5 °C. The dead time was estimated to be 3.1 ms. The full detector range intensity was integrated with an exposure time per data point of 0.15 s at 11.1 °C, 15.1 °C, and 22.1 °C; 0.05 s at 27.5 °C; and 0.03 s at 32.5 °C. SANS data (DOI: 10.5291/ILL-DATA.8-03-872) were collected using a 2D detector and reduced to 1D using the reduction package GRASP[13]. At each temperature, at least five traces were averaged and analysed by nonlinear least-squares fitting[14].

Kinetics of phospholipid exchange among SMALPs

The transfer of lipid molecules among nanoparticles can take place through (i) desorption and interparticle diffusion of lipid monomers through the aqueous phase[15–17] and (ii) lipid exchange through particle collisions[18–21]. If the particles making up the

two populations that exchange lipid molecules are identical in size and shape, the observed rate constant resulting from monomer diffusion takes the form[18,19,21]:

$$k_{\mathrm{obs,dif}}\,(c_{\mathrm{L}}) = \frac{k_{\mathrm{dif}} c_{\mathrm{L}}}{c_{\mathrm{L}}^{\circ} + c_{\mathrm{L}}} \tag{1}$$

where k_{dif} is the diffusional exchange rate constant and c_{L}° and c_{L} are the bulk solution concentrations of lipid in the donor and acceptor populations, respectively. For second-order ("bimolecular") collision-dependent lipid transfer, the observed rate constant reads:

$$k_{\mathrm{obs,col}}\,(c_{\mathrm{L}}) = k_{\mathrm{col}} c_{\mathrm{L}} \tag{2}$$

where k_{col} is the second-order collisional exchange rate constant. If both of the above processes are at play, the overall observed rate constant is given by the sum of equation (1) and (2)[18,19,21]:

$$k_{\mathrm{obs}}\,(c_{\mathrm{L}}) = \frac{k_{\mathrm{dif}} c_{\mathrm{L}}}{c_{\mathrm{L}}^{\circ} + c_{\mathrm{L}}} + k_{\mathrm{col}} c_{\mathrm{L}} \tag{3}$$

Temperature-dependent TR-SANS decays

Upon mixing h- and d-SMALPs, lipid exchange leads to an exponential decrease in the SLD with time, as given by:

$$I(t) = I_{\infty} + e^{-k_{\mathrm{obs}} t}(I_0 - I_{\infty}) \tag{4}$$

where $I(t)$ is the signal intensity at time t, I_0 and I_{∞} are the initial and final intensities, respectively. According to transition-state theory[22], the second-order collisional rate constant can be expressed as a function of temperature, T, according to:

$$k_{\mathrm{col}}(T) = \frac{1}{\mathrm{M}} \frac{RT}{N_{\mathrm{A}} h} e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R} \tag{5}$$

with R being the universal gas constant, N_{A} Avogadro's number, h Planck's constant, and $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ the activation enthalpy and entropy, respectively. M denotes the unit "molar", with c_{L} = 1 M being the standard concentration. At the c_{L} values used for TR-SANS, the relative contribution of monomer diffusion to the overall lipid transfer rate is very small, so that the first term on the right-hand side of equation (3) can be neglected. Thus, insertion of equation (5) into equation (3) and further into equation (4) yields:

$$I(t) = I_{\infty} + e^{-t\left((RT/N_{\mathrm{A}}h) e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R}\right) c_{\mathrm{L}}/\mathrm{M}} (I_0 - I_{\infty}) \tag{6}$$

In this global fitting equation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are treated as global fitting parameters, whereas I_0 and I_{∞} are local (i.e., T-specific) fitting parameters. Best-fit parameter values and 95% confidence intervals were derived by nonlinear least-squares fitting in Excel spreadsheets[14].

The entropic component at arbitrary c_{L} values is related to the above standard-state value at c_{L} = 1 M through:

$$-T\Delta S^{\ddagger}(c_{\mathrm{L}}) = -T\Delta S^{\ddagger}(1\ \mathrm{M}) - RT\ln(c_{\mathrm{L}}/1\ \mathrm{M}) \tag{7}$$

The apparent values of the molar activation enthalpy and entropy were corrected for the temperature dependence of the buffer viscosity as detailed elsewhere[23].

Results

Time-resolved small-angle neutron scattering (TR-SANS) relies on monitoring changes in the neutron scattering length density (SLD) accompanying the exchange of molecules between hydrogenated and deuterated particles. This principle has been used to lipid transfer among MSP nanodiscs[24] or LUVs[25]. We prepared SMALPs that harboured either hydrogenated or deuterated DMPC (h- and d-SMALPs, respectively) in Tris buffer containing 42.8% (*v*/*v*) D_2O, which matches the SLD of nanodiscs composed of equal amounts of h- and d-DMPC (h/d-SMALPs). We then mixed equal volumes of the two SMALP preparations to reach a final concentration of 10 mM of each of the two lipids. The initially strong total SLD resulting from coexisting populations of h- and d-SMALPs rapidly decayed after mixing, with a monotonic decrease in decay time with increasing temperature (Fig. 1a). Each decay was fitted individually to yield temperature-dependent k_{obs} values (equation (4)). On raising the temperature from 11 °C to 33 °C, we thus observed a tenfold increase in k_{obs} (Fig. 1b).

Since collisional lipid exchange dominates at c_{L} = 10 mM, monomer diffusion can be neglected to a very good approximation. Thus, the TR-SANS results can be interpreted in terms of a collisional process with a second-order rate constant given by $k_{\mathrm{col}} \approx k_{\mathrm{obs}}/c_{\mathrm{L}}$ (equation (3)). Using a global fit based on transition-state theory[22] (equation (5) and (6)), we analysed the temperature dependence of k_{col} to characterise the collisional transition state (Fig. 1c). This allowed a comparison with MSP nanodiscs[24] and LUVs[25], which exhibit lipid transfer solely by monomer diffusion. For both of the latter, the pronounced enthalpic penalty incurred upon formation of the transition state can be ascribed to the disruption of dispersive acyl chain and polar headgroup interactions[19,21]. In agreement with this interpretation, $\Delta H^{\ddagger}$ is substantially lower for SMALPs. While the entropic term is marginally favourable for MSP nanodiscs, it is moderately unfavourable for LUVs. For a second-order

collisional process, $-T\Delta S^{\ddagger}$ obviously depends on c_L (equation (7)). Within the experimentally relevant c_L range, we found the $-T\Delta S^{\ddagger}$ values of SMALPs to be small in magnitude and, thus, similar to that of MSP nanodiscs and more favourable than that of LUVs. Consequently, $\Delta G^{\ddagger}$ is more favourable for SMALPs than for either of the other two membrane mimics.

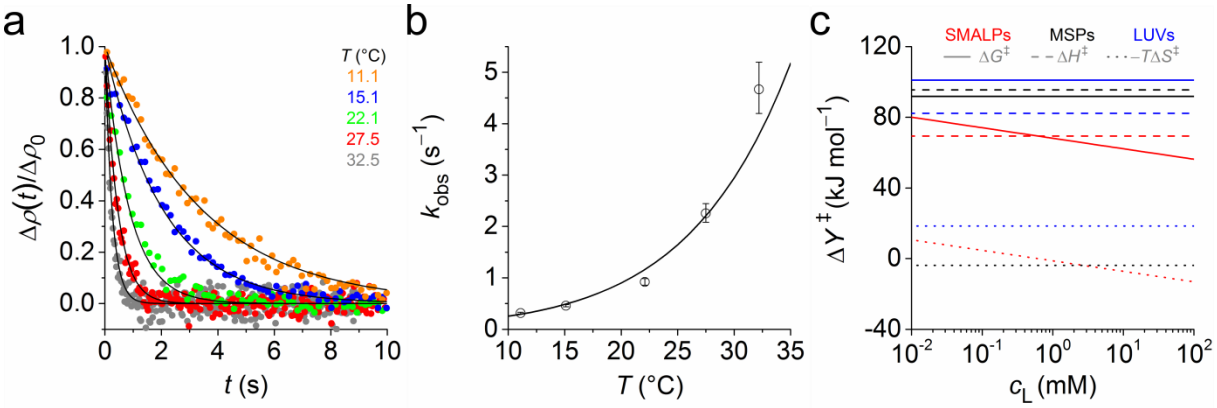

Figure 1 Transfer of DMPC among SMALPs as monitored by TR-SANS at various temperatures. (a) Decay of normalised SLD, $\Delta\rho(t)/\Delta\rho_0$, with time, t, upon mixing d- and h-SMALPs at 10 mM of each lipid and different temperatures, T. Shown are experimental data (coloured dots) and a global fit (solid lines) according to equation (6). (b) Lipid exchange rate constants, k_{obs}, derived from local fits to data in panel a according to equation (5) and results from a global fit (solid line) according to equation (6). Error bars are 95% confidence intervals of local fits. (c) Changes in molar quantities of activation, $\Delta Y^{\ddagger}$, at 37 °C for the Gibbs free energy, $\Delta G^{\ddagger}$, enthalpy, $\Delta H^{\ddagger}$, and entropy, $-T\Delta S^{\ddagger}$, as obtained from equation (6) and (7) and data in references 24 and 25.

Their highly dynamic lipid-exchange behaviour sets SMALPs apart from other bilayer-based membrane mimics such as MSP nanodiscs and LUVs, which has profound implications for the interpretation of membrane-protein studies relying on the unique properties of SMA. On the one hand, it has been found[10,26] that protein-containing SMALPs isolated from native membranes are enriched in certain lipid species as compared with protein-free SMALPs. The present finding that SMALPs quickly exchange lipids then means that this enrichment must result from preferential protein/lipid interactions that are preserved in SMALPs, where they are sufficiently strong to retain these lipids in the immediate vicinity of the protein. In other words, the SMALPed "lipidome" is not merely a snapshot[27] of the situation in the original membrane at the time of solubilisation but reflects rather strong protein/lipid interactions. Conversely, it seems plausible that weaker protein/lipid interactions, although present in the membrane before solubilisation, could be lost even after SMALP formation. On the other hand, an exciting perspective offered by fast interparticle lipid exchange lies in the possibility of systematically investigating the effects of various lipids on the structures, dynamics, and functions of SMALPed membrane proteins, which can easily be accomplished by changing the overall lipid composition through addition of SMALPs containing other types of lipids.

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