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Kinetics of lipid mixing between bicelles and nanolipoprotein particles

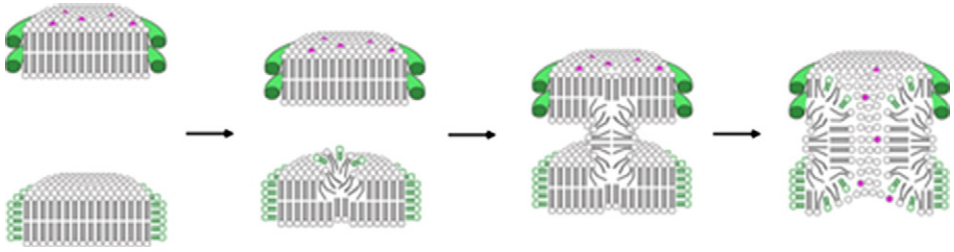
Ginny Lai [a,1], Kevin Muñoz Forti [b], Robert Renthal [a,c,*]

[a] Biology Department, University of Texas at San Antonio, San Antonio, TX 78249, USA
[b] Biology Department, University of Puerto Rico, Ponce, PR, USA
[c] Biochemistry Department, University of Texas Health Science Center, San Antonio, TX 78229, USA

HIGHLIGHTS

- Nanolipoprotein particle lipid mixing kinetics with bicelles was slow and sigmoidal.
- Short-chain lipids mixed rapidly with nanolipoprotein particles.
- Added apolipoprotein had little effect on the kinetics.
- The reaction had a high activation energy.
- Nanolipoprotein particle lipids may mix with bicelles via fusion pores.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanolipoprotein particles (NLPs), also known as nanodiscs, are lipid bilayers bounded by apolipoprotein. Lipids and membrane proteins cannot exchange between NLPs. However, the addition of bicelles opens NLPs and transfers their contents to bicelles, which freely exchange lipids and proteins. NLP–bicelle interactions may provide a new method for studying membrane protein oligomerization. The interaction mechanism was investigated by stopped flow fluorometry. NLPs with lipids having fluorescence resonance energy transfer (FRET) donors and acceptors were mixed with a 200-fold molar excess of dihexanoyl phosphatidylcholine (DHPC)/dimyristoyl phosphatidylcholine (DMPC) bicelles, and the rate of lipid transfer was monitored by the disappearance of FRET. Near or below the DMPC phase transition temperature, the kinetics were sigmoidal. Free DHPC and apolipoprotein were ruled out as participants in autocatalytic mechanisms. The NLP–bicelle mixing rate showed a strong temperature dependence (activation energy = 28 kcal/mol). Models are proposed for the NLP–bicelle mixing, including one involving fusion pores.

1. Introduction

Small lipid bilayer discs are widely used to study integral membrane proteins in a membrane-like environment. Bicelles are mixtures of bilayer-forming phospholipids combined with detergents that cover the exposed lipid hydrocarbon chains of the bilayer edge. The detergents can be either micelle-forming short-acyl-chain lipids or bile salts. Bicelles are polymorphic, but when the bilayer lipid to detergent mole ratio (q) is less than ~1, bicelles consist of bilayer discs [1,2]. Nanolipoprotein particles (NLPs), also known as nanodiscs, are bilayer discs with apolipoproteins covering the hydrocarbon edges [3,4]. Membrane proteins and lipids can exchange between bicelles, but they cannot move from one NLP to another, presumably because of the apolipoprotein barrier. However, the addition of bicelles to NLPs opens them, and the NLP lipids and proteins transfer to bicelles [5]. Membrane proteins can be inserted into NLPs by cell-free protein synthesis [6]. By manipulating steric and stoichiometric factors in cell-free protein synthesis, it should be possible to prepare predominantly

* Corresponding author at: Department of Biology, University of Texas at San Antonio, 1 UTSA Circle, San Antonio, TX 78249, USA. Tel.: +1 210 458 5452.
E-mail address: robert.renthal@utsa.edu (R. Renthal).
[1] Present address: Department of Medicine, University of Florida College of Medicine, Gainesville, FL 32610, USA.

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monomeric membrane proteins in NLPs. Thus, the bicelle-induced transfer process may provide a new method for studying membrane protein oligomerization [5].

The mechanism of the interaction between NLPs and bicelles is unknown. Because bicelles are in equilibrium with detergent monomers [1,7], trace amounts of mixed micelles could transport NLP lipids and proteins to bicelles. Alternatively, the NLP apolipoprotein, in a contracted conformation [8], might transfer NLP lipids and proteins to bicelles. We have now tested the effects of bicelle detergent, apolipoprotein, and temperature on lipid transfer from NLPs to bicelles, using stopped-flow kinetics.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD PE), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (LR-PE) were obtained from Avanti Polar Lipids (Alabaster, AL). MSP1E3D1, the NLP-forming apolipoprotein, was prepared as previously described [5] or obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Bicelle and NLP preparation

Bicelles and NLPs were prepared as previously described [5]. Fluorescent lipids were incorporated into NLPs at 0.02 to 0.05 mole fraction. NLPs were purified on a Superdex 200 10/30 column (GE Healthcare, Piscataway, NJ). Final concentrations were determined by measuring absorbance spectra on an Aviv/Cary 14 spectrophotometer (Aviv Biomedical, Lakewood, NJ). Extinction coefficients used were: NBD, $2.1 \times 10^4\ M^{-1}\ cm^{-1}$ at 460 nm; LR, $7.5 \times 10^4\ M^{-1}\ cm^{-1}$ at 566 nm [9,10]; and MSP1E3D1, $2.94 \times 10^4\ M^{-1}\ cm^{-1}$ at 280 nm (http://sligarlab.life.uiuc.edu/nanodisc/protocols.html). In NLPs containing fluorescent lipids, the MSP1E3D1 concentration was measured after subtracting the 280 nm absorbance due to NBD and LR as follows: NBD 280 nm absorbance was 0.086 times the 460 nm absorbance [11] and LR 280 nm absorbance was 0.139 times the 566 nm absorbance (sulforhodamine B, www.fluorophores.tugraz.at/fluorescence).

2.3. Stopped flow fluorometry

NLPs containing NBD-PE and LR-PE were mixed with DMPC/DHPC bicelles (q = 1) in an SFM-20 stopped flow spectrometer (Bio-Logic, Knoxville, TN). The excitation light, at 440 nm with a 10 nm slit, was from a QM4 fluorometer (PTI, Edison, NJ), via a fiber optic cable. The dequenched NBD emission was detected by an R376 photomultiplier (Hamamatsu, Bridgewater, NJ), with a 520 nm interference filter, 10 nm bandwidth (Newport Corp., Irvine, CA). NLPs were loaded into a 10 mL syringe (syringe 1) at 0.6 μM or 1.2 μM in phosphate-buffered saline (PBS, 0.137 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) or 4.2 μM in tris buffer (20 mM tris, 0.1 M NaCl, pH 7.4). Bicelles were loaded into a 1 mL syringe (syringe 2) at 90 mM DMPC and 90 mM DHPC in PBS or 45 mM DMPC and 45 mM DHPC in tris buffer. Each reaction was 147 μL, mixed at a 2:1 ratio of syringe 1 to syringe 2, flowing at 1 mL/s into an FC-15 (1.5 mm pathlength) cuvette. Under these conditions, the instrument dead time was 37 ms. The syringe holders, mixing head and cuvette were held at constant temperature with a Haake K10 water bath and DC10 circulator (Thermo Fisher, Waltham, MA). The difference between the bath and syringe temperature was determined by using a thermocouple. The effect of temperature on the reaction rate was measured in 4 °C increments. The stopped flow apparatus was equilibrated for 25 min at each new temperature before making measurements.

3. Results

3.1. NLP reaction with bicelles

NLPs containing fluorescence resonance energy transfer (FRET) donor–acceptor pairs of fluorescent-tagged lipids (NBD-PE and LR-PE) were mixed with a large excess of bicelles (q = 1). The time dependence of the increase in dequenched NBD fluorescence (Fig. 1) was assumed to measure the rate of lipid transfer from NLPs and subsequent further transfer between bicelles. The data were fit with an empirical equation:

$$y = A(1 - \exp(-kt))^n \quad (1)$$

where y is the NBD fluorescence at time t, A is the maximum dequenched NBD fluorescence signal when all the lipids are dispersed in bicelles (proportional to the total concentration of NBD-PE), k is the pseudo-first-order rate constant, and n is a fitted parameter. The rate constant was surprisingly small ($0.26\ s^{-1}$, lower curve, Fig. 1). This can be compared with the expected pseudo-first-order rate constant for a simple NLP–bicelle collision mechanism. The measured diffusion coefficient for bicelles [12] is $4 \times 10^{-7}\ cm^2/s$. Combined with the measured radii of bicelles and NLPs [5], this suggests a second-order rate constant in the range of $10^9\ M^{-1}\ s^{-1}$ [13] for a reaction mechanism with the rate-limiting step for lipid transfer involving simple collisions between NLPs and bicelles. Since the bicelle concentrations (≈0.2 mM) were in large excess over the NLP concentrations (≈1 μM), the expected pseudo-first-order rate constant for a collision mechanism would be in the range of $2 \times 10^5\ s^{-1}$.

The kinetic curves displayed a sigmoid shape, which Eq. (1) represents by using the exponent n (n = 1.6 in Fig. 1). Sigmoid reaction kinetics can indicate an autocatalytic mechanism. Eq. (1) fits the experimental data with correlation coefficients greater than 0.99. However, when very high concentrations of NLPs were used (Fig. 1, upper curve), a burst phase was observed in the first several hundred milliseconds. The magnitude of the burst phase was less than 4% of the final NBD fluorescence. Thus it could not be observed at low concentrations, where there was a lower signal-to-noise ratio. The properties of the burst phase were not pursued in the experiments reported here.

Initial rates of fluorescent lipid mixing were estimated from Fig. 1 and are given in Table 1. When the NLP concentration was doubled,

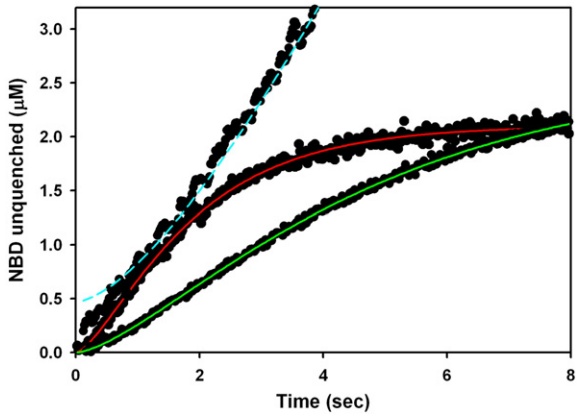

Fig. 1. Kinetics of bicelle-induced opening of nanolipoprotein particles (NLPs), measured by the disappearance of fluorescence resonance energy transfer between lipids. Conditions (final concentrations): Upper: 2.8 μM NLPs, 22.9 μM 1,2-dimyristoyl-*sn*-glycerol-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (LR-PE), 29.3 μM 1,2-dimyristoyl-*sn*-glycerol-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE), 30 mM bicelle lipid; middle: 0.4 μM NLPs, 2.6 μM LR-PE, 2.1 μM NBD-PE, 60 mM bicelle lipid; lower: 0.8 μM NLPs, 2.5 μM LR-PE, 2.6 μM NBD-PE, 60 mM bicelle lipid. Lines (Eq. (1) parameters): red, $k = 0.66\ s^{-1}$, n = 1.6; green, $k = 0.26\ s^{-1}$, n = 1.6; blue, $k = 0.07\ s^{-1}$, n = 1.6. Temperature, 23.6 °C.