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Apolipoprotein C-III Nanodiscs Studied by Site-specific Tryptophan Fluorescence

Chase A. Brisbois and Jennifer C. Lee*

Laboratory of Protein Conformation and Dynamics, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

Abstract

Apolipoprotein C-III (ApoC-III) is found on high-density lipoproteins (HDL) and remodels 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine vesicles into HDL-like particles known as nanodiscs. Using single-Trp containing ApoC-III mutants, we have studied local sidechain environments and interactions in nanodiscs at positions W42, W54, and W65. Using transmission electron microscopy and circular dichroism spectroscopy, nanodiscs were characterized at the ultrastructural and secondary conformational levels, respectively. Nearly identical particles (15 ± 2 nm) were produced from all proteins containing approximately 25 ± 4 proteins per particle with an average helicity of 45–51% per protein. Distinct residue-specific fluorescence properties were observed with W54 residing in the most hydrophobic environment followed by W42 and W65. Interestingly, time-resolved anisotropy measurements revealed that Trp sidechain mobility is uncorrelated to the polarity of its surroundings. W54 is the most mobile compared to W65 and W42, which are more immobile in a nanodisc-bound state. Based on Trp spectral comparisons of ApoC-III in micellar and vesicle environments, ApoC-III binding within nanodiscs more closely resembles a bilayer-bound state. Despite being structurally similar, we found marked differences during nanodisc formation by the Trp variants as a function of temperature with W42 behaving the most similar to the WT protein. Our data suggest that despite the modest mutations of Trp to Phe at two of the three native sites, the interfacial location of W42 is important for lipid binding and nanodisc assembly, which may be biologically meaningful as of the three Trp residues, only W42 is invariant among mammals.

High-density lipoproteins (HDL) are structurally dynamic particles that are responsible for the reverse transport of cholesterol from peripheral tissues back to the liver.^{1, 2} Apolipoprotein C-III (ApoC-III) is a 79 amino acid protein component of HDL³ as well as other lipoproteins.⁴ ApoC-III plays an important role in the uptake of lipoproteins by the liver and is an inhibitor of lipoprotein lipase.⁵ Not surprisingly, mutations and dysregulation of ApoC-III have been implicated in certain dyslipidemic conditions. For example, overproduction of ApoC-III in patients has been correlated to hypertriglyceridemia, a major risk factor for atherosclerotic cardiovascular disease.⁶ A naturally occurring ApoC-III

*Corresponding Author leej4@mail.nih.gov.

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SUPPORTING INFORMATION AVAILABLE

Figure S1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

missense mutation in humans (K58E) leads to hypotriglyceridemia potentially caused by disrupting the C-terminal lipid binding domain.⁷ Other studies also have correlated HDL-bound ApoC-III as a potential indicator of cardiovascular disease risk.^{8, 9} However, despite its prevalence in health and disease, very little is known about how ApoC-III binds to HDL particles.

Reconstituted HDL particles, also called “nanodiscs,” are unique protein-lipid complexes produced *in vitro* that resembles the structure of nascent HDL. Nanodiscs are involved in a wide range of active research in basic HDL biology and applications.¹⁰⁻¹³ While the bulk of structural studies have centered around nanodiscs composed of apolipoprotein A-I (ApoA-I),^{14, 15} the major protein component of HDL, other apolipoproteins, including ApoC-III, have been reported to spontaneously form nanodiscs.¹²

A well-established ApoC-III nanodisc is composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and was first characterized under negative stain transmission electron microscopy (TEM) by Hoff *et al.*, revealing a striking resemblance to HDL.¹⁶ Pownall *et al.* observed that complexes of DMPC and ApoC-III were formed between a lipid-to-protein molar ratio (L/P) of 35 and 110.¹⁷ Based on sedimentation velocity experiments, Aune *et al.* suggested that the ApoC-III/DMPC complexes were asymmetrical, either a prolate or oblate ellipsoid.¹⁸ Following this work, the ultrastructure of the ApoC-III/DMPC complexes was studied by small-angle X-ray scattering and was determined to be oblate ellipsoids, approximately 17 nm wide and 5 nm thick.¹⁹ However, since it has been established that ApoC-III adopts a helical structure upon interacting with phosphatidylcholine,²⁰ there has been little recent work on elucidating the conformation of ApoC-III in nanodiscs. It remains to be determined whether the protein primarily binds to the edge¹⁹ or the face²¹ of the disc.

Currently, the only known lipid-bound model of ApoC-III uses sodium dodecyl sulfate (SDS) micelles.²² In this state, ApoC-III adopts six, curved amphipathic helices that wrap around the spherical micelle surface. While SDS is similar in size, nanodiscs are anisotropic particles primarily composed of phospholipids. Differences in shape and lipid structure may significantly alter the binding preferences of ApoC-III and the conformation it adopts. Therefore, it is necessary to study ApoC-III/DMPC nanodiscs directly to advance our knowledge of ApoC-III lipid binding and give a more complete understanding of the impact of ApoC-III in HDL structure.

Herein, we describe the synthesis, purification, and characterization of ApoC-III/DMPC nanodiscs. We chose to focus our study on nanodiscs produced at a L/P ~50, previously characterized by Laggner *et al.*¹⁹ and Pownall *et al.*¹⁷ The formation of nanodiscs were confirmed by TEM and the global protein secondary structural change from disordered, lipid-free to α -helical nanodisc-bound ApoC-III was monitored by circular dichroism (CD) spectroscopy. To gain site-specific information on the nanodisc-bound ApoC-III, we turned to measurements of intrinsic Trp fluorescence, as the emission of Trp is highly sensitive to its local environment,^{23, 24} and has been proven to be a lipid-sensitive probe in membrane protein studies.^{25, 26} Because human ApoC-III has three native Trp residues located at positions 42, 54, and 65 (Figure 1), to decouple individual contribution from each site, we

have employed three single-Trp mutants of ApoC-III, abbreviated as W42 for W54F/W65F, W54 for W42F/W65F, and W65 for W42F/W54F, which we have previously used to study ApoC-III binding to anionic phospholipids.²⁷

The effects of Trp mutations on nanodisc formation kinetics were measured using a turbidity assay. Infrared (IR) and CD spectroscopy were used to identify and evaluate the chemical composition and protein secondary structure of the nanodiscs, respectively. In addition, polydispersity of particle size and absolute lipid content were evaluated using dynamic light scattering (DLS) measurements and a chemical phosphorus assay. At each Trp site, time-resolved anisotropy and steady-state fluorescence were measured to report on changes in the mobility of the Trp sidechain and the local polarity of each Trp surrounding. Finally, steady-state fluorescence measurements of micelle- and vesicle-bound ApoC-III were carried out for comparison to discern unique structural features of nanodiscs as well as to examine the consequences of lipid structure and size on lipid-bound ApoC-III conformation.

MATERIALS AND METHODS

Materials

Sodium chloride (99.5%), sodium phosphate monobasic (99%), sodium phosphate dibasic (99%), tris(hydroxymethyl)aminomethane (Tris) (99.9%), ethylenediaminetetraacetic acid (EDTA) (99%), N-acetyl-L-tryptophanamide (NATA), L-ascorbic acid (99.0%), hydrogen peroxide solution (30%), ammonium molybdate (99.98%), and sodium phosphate dibasic standard (1000 ± 4 mg/L) were purchased from Sigma-Aldrich (St. Louis, MO). The lipids: 1-palmitoyl-2-oleoyl-*sn*-glycero-phosphocholine (POPC) (> 99%), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (> 99%), 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPC) (> 99%) and n-dodecyl-β-D-maltoside (DDM) (> 99%) were purchased from Avanti Polar Lipids (Alabaster, AL). All chemicals were used as received.

Recombinant Protein Purification

The original ApoC-III expression plasmid (pET23b) was provided by Philippa Talmud (University College London Medical School, London, UK).²⁸ This construct contains a C-terminal hexa-histidine tag with a preceding two-residue (LE) spacer. Site-directed mutagenesis, *E. coli* expression, and protein purification have been described previously.^{27, 29} Briefly, frozen cell pellets (120 g) obtained from a 14-L fermentor growth were resuspended in 300 mL of lysis buffer (100 mM NaPi, 100 mM NaCl, 8 M urea, pH 7.4) supplemented with 6 protease inhibitor cocktail tablets (Roche 04693159001) and stirred for 60 min at RT. The cells were homogenized and passed twice through a microfluidizer (M-110PS, Microfluidics) at ~18,000 psi. Cellular debris was removed by centrifugation at 12,500 *g* for 2 h. The supernatant was collected and 20 mL of cobalt TALON metal affinity resin (Clontech) was added. The protein solution was shaken overnight at 160 rpm and 4 °C using an Innov 4230 refrigerated incubating shaker. The resins were separated, washed, and the protein solution was eluted using vacuum filtration. The resin was washed 4 times with 50 mL of lysis buffer and eluted with 80 mL of lysis buffer supplemented with 300 mM imidazole over two steps (60 mL followed by 20 mL).

The protein solution (~80 mL) was concentrated to ~2 mL using Amicon Ultracel MWCO 3 kDa filters (Millipore). Further purification was achieved by gel filtration chromatography on a HiLoad 16/60 Superdex 75 (GE Healthcare) column followed by anionic exchange chromatography using a Mono Q HR 16/10 (Pharmacia) column. ApoC-III was eluted using a linear salt gradient (50–140 mM NaCl). Chromatography was carried out at 4 °C and each column was equilibrated with identical buffer (10 mM NaPi, 0.8 M urea, pH 7.4).

Protein was detected by His-tag staining. SDS-PAGE gels were transferred to a PVDF membrane (Invitrogen iBlot® Gel Transfer). The membrane was shaken gently in 1% (w/v) BSA and 1X TBST (50 mM Tris, 150 mM NaCl, 1% Tween 20, pH = 7.6; KPL, Inc.) for 45 min followed by the addition of 3 µL of HisDetector™ Nickel-HRP (KPL, Inc.) and shaken for a further 45 min. Afterwards, the membrane was placed in 1X TBST and incubated for 5 min (repeated 3 times with fresh TBST). Finally, the membrane was placed in TMB peroxidase substrate solution. The reaction was stopped after 15 min by rinsing the membrane with ddH₂O. Fractions containing only ApoC-III were pooled, concentrated to 50–100 µM (molar extinction coefficient, $\epsilon = 19,480 \text{ M}^{-1} \text{ cm}^{-1}$ for WT, $\epsilon = 8,480 \text{ M}^{-1} \text{ cm}^{-1}$ for single-Trp mutants) using 3 kDa Amicon filters, flash-frozen in liquid nitrogen, and stored at –80 °C. Molecular weights were determined by electrospray ionization mass spectrometry which revealed two primary components of ApoC-III with and without an N-terminal methionine, 9829 and 9960 Daltons, respectively (NHLBI Biochemistry Core). Prior to experiments, ApoC-III is thawed on ice and exchanged into filtered (0.22 µm) buffer using a PD-10 column (GE Healthcare).

Preparation of Multilamellar DMPC Vesicles

DMPC lipids were transferred to a glass vial and dried from chloroform under flowing nitrogen gas to create an even film on the glass surface. The vial was placed in a vacuum oven (< 20 kPa) at 40 °C for at least 2 h. The dried DMPC lipids were resuspended with 1 mL of NaPi or Tris buffer (10 mM NaPi or Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) to a concentration of 4 mM. Multilamellar vesicles (MLVs) were formed by vortexing (Scientific Industries Vortex-Genie 2, set to 8) for three repetitions of 1 min each followed by a 5 min equilibration period at 28 °C. The lipid solutions were used within 1 h of preparation.

Preparation of Extruded POPC Vesicles

POPC lipids were transferred to a glass vial and dried from chloroform under flowing nitrogen gas to create an even film on the glass surface. The vial was placed in a vacuum oven (< 20 kPa) at 40 °C for at least 2 h. The lipids were rehydrated with buffer to a concentration of 10–20 mM and resuspended by vortexing (Scientific Industries Vortex-Genie 2, set to 8) for three repetitions of 1 min each followed by a 5 min equilibration period at RT. After one freeze-thaw cycle, the lipids were extruded through a 100 nm pore membrane (Millipore) 5 times at 200 psi followed by 5 more extrusions through a 30 nm pore membrane (Millipore) at 500 psi using a LIPEX 10 mL thermobarrel extruder ($\langle r \rangle = 30 \text{ nm}$, $P_d = 10\%$). Vesicles were used within two days of preparation. Vesicles are stable (< 10% deviation in mean radius based on DLS) up to two weeks after preparation.

Optical Clearance Assay

WT and single-Trp mutant ApoC-III solutions (10 mM NaPi, 100 mM NaCl, 1 mM EDTA, pH 7.4) were incubated in a quartz cuvette in a HP8453 spectrophotometer (Hewlett-Packard) at 28 °C for 10 min. DMPC MLVs kept at RT were added (5% of total solution volume) to the cuvette and quickly pipetted 20 times to mix. Absorbance at 500 nm was recorded every 2 s for 30 min using HP 845x UV-Visible ChemStation in kinetics mode (NHLBI Biophysics Core).

Transmission Electron Microscopy

TEM grids (400-mesh Formvar carbon coated copper, Electron Microscopy Sciences) were glow discharged in a PLECO® easiGlow for 60 s at 15 mA and 0.39 mBar. Samples (8 µL) were incubated on grids for 1 min. After incubation, the sample solution was wicked with filter paper. Two or three drops of 1% (w/v) aqueous uranyl acetate were added. Excess uranyl acetate was immediately wicked off with filter paper and the grids were air dried. The grids were imaged using a JEOL JEM 1200EX transmission electron microscope (80 keV) equipped with an AMT XR-60 digital camera (NHLBI EM Core).

Preparation of Nanodiscs

DMPC MLVs (~1.5 mM) and ApoC-III (~30 µM) were combined at RT to a lipid-to-protein molar ratio of 50 and incubated at 28 °C for 2 h using a water bath. A Tricorn Superdex 200 10/300 GL column (GE Healthcare) equilibrated with either NaPi or Tris buffer (4 °C) was then used to separate the nanodiscs from free protein in the sample. The sample was concentrated to ~200 µL using 3 kDa Amicon filters and filtered through a 0.22 µm filter onto a 100 µL loop. The fractions containing nanodiscs were stored at 4 °C and used for experiments within 48 h.

Dynamic Light Scattering

Purified nanodiscs were filtered through 0.22 µm membrane into a disposable plastic cuvette (Eppendorf, Uvette®, 220–1600 nm). Light scattering was measured on a DynaPro NanoStar (Wyatt Technology Corp) using DYNAMICS control software (version 7.0.2.7, NHLBI Biophysics Core). Every measurement was the average of at least 10 acquisitions (5 s) with greater than 100,000 counts/second (typically ~300,000) at 28 °C. Samples were measured directly off the gel filtration column.

Fourier Transform Infrared Spectroscopy

Samples were concentrated in an Amicon Ultracel MWCO 3 kDa filter (~200 µM) at 4 °C. Measurements were taken in solution (10 µL) at RT using a Nicolet iS50 spectrophotometer (Thermo Scientific) by attenuated total reflectance (HARRICK, ConcentratIR 2). Each nanodisc spectrum was buffer subtracted. Background spectra were taken of clean Si crystal before each measurement. The crystal was cleaned with ddH₂O followed by isopropanol to remove salts and lipids, respectively.

Circular Dichroism Spectroscopy

Measurements were made on a Jasco J-715 spectropolarimeter (NHLBI Biophysics Core). Purified nanodiscs were measured in a 4×10 mm fluorescence quartz cuvette (Starna Cells, cat. no. 29-Q-10) oriented such that light passes through the 10-mm side to achieve a 4-mm path length. At least five accumulated scans were collected and averaged using the following settings: 200 – 260 nm, 1 nm bandwidth, 1 nm steps, 50 nm/min scan rate, 0.5-s integration time, and 28 °C. Temperature scan experiments were performed by first incubating 950 μ L of 10 μ M protein in buffer (10 mM NaPi, 100 mM NaCl, 1 mM EDTA, pH 7.4) at 45 °C for 15 min. After adding RT DMPC MLVs (4 mM, 50 μ L), the solution was mixed immediately by pipetting and data were collected with the following settings: 1 nm bandwidth, 20 °C/h scan rate, and 0.5 °C data spacing. Buffer baseline subtractions were applied to all spectra and scans. The mean residue ellipticity (MRE, $[\Theta]$) was calculated according to eq 1.³⁰

$$[\Theta] = \frac{100\theta}{nlc} \quad (1)$$

where θ is the measured ellipticity signal (mdeg), n is the number of residues in the protein, l is the path length (cm) and c is the concentration of the protein (mM). Fractional helicity was calculated using eq 2.³⁰

$$f_{helix} = \frac{[\Theta]_{222} - [\Theta]_{coil}}{[\Theta]_{helix} - [\Theta]_{coil}} \quad (2)$$

where $[\Theta]_{222}$ is the MRE at 222 nm, $[\Theta]_{coil} = 640 - 45T$, $[\Theta]_{helix} = -40,000(1 - 2.5/n) + 100T$, n is the number of residues in the protein and T is the temperature (°C).

Determination of Total Phosphorus

Three reagent solutions were prepared prior to experiment. H₂SO₄ solution (8.9 N) was prepared by slowly adding 100 mL of concentrated H₂SO₄ into 300 mL of ddH₂O and stored at RT. L-ascorbic acid solution (10% w/v) was prepared by dissolving 5.0 g of L-ascorbic acid into ddH₂O up to 50 mL and stored at 4 °C for up to one month. Ammonium molybdate(IV) solution (2.5% w/v) was prepared by dissolving 1.25 g of molybdate into ddH₂O to a volume of 50 mL and stored at 4 °C for up to one month. A one-tenth dilution of the sodium phosphate dibasic standard (100 mg/mL) was added to six glass test tubes (13 $\times$ 100 mm) with the following volumes: 0 (blank), 50, 100, 150, 250, and 350 μ L. At least three different volumes (50–400 μ L) of each nanodisc solution were added to the remaining test tubes. Twenty test tubes were measured per assay. To each test tube, boiling stones (PTFE, Chemware) and 8.9 N H₂SO₄ solution (450 μ L) were then added. The test tubes were placed on a metal block that can hold twenty test tubes and placed on a hot plate. The test tubes were heated to 200–240 °C (visible boiling) for 25 min. The block and test tubes were insulated with aluminum foil to promote even heating. Test tubes were removed from the block and cooled for 5 minutes at RT. Hydrogen peroxide (150 μ L) was added to each test tube before they were heated again to 200–240 °C for 30 min. After heating, the test

tubes were cooled for 15 min at RT and ddH₂O (3.9 mL) was added to each tube. One volume each (500 μ L) of 2.5% ammonium molybdate and 10% ascorbic acid were added consecutively to every test tube with gentle vortexing after each addition. All tubes were heated on the block for 7 min at 90–120 °C. The test tubes were cooled down to RT and their absorbance at 820 nm was measured relative to the blank sample in polystyrene disposable cuvettes (VWR, cat. no. 97000-586) on a Cary 300 Bio spectrophotometer (2.0 s avg. time, 1.5 nm spectral bandwidth). This procedure was modified from www.avantilipids.com.^{31, 32}

Steady-state Fluorescence Measurements

Tryptophan fluorescence measurements were taken on a Horiba Fluorolog-3 spectrofluorometer using an excitation wavelength of 295 nm, an emission range of 300–500 nm, 1 nm steps, 0.3-s integration time, and excitation and emission slit widths of 1 and 2 nm, respectively. Data sets were calibrated to water raman peak at 397 nm (0.5 nm step accuracy). All measurements were taken at 28 °C with protein concentrations of 3–10 μ M using a quartz cuvette (Starna Cells, cat. no. 21-Q-2). For all spectra, buffer background scans were collected and subtracted. Corrected spectra were fit to a lognormal distribution according to eq 3.²³

$$I(\lambda) = I_o \exp \left[-\frac{\ln(2)}{\ln^2(\rho)} \ln^2 \left(1 + \frac{(\lambda - \lambda_{max})(\rho^2 - 1)}{\rho\Gamma} \right) \right] \quad (3)$$

where I_o , λ_{max} , Γ , and ρ represent maximum spectral intensity, wavelength at maximum intensity, spectra with at half-maximum intensity, and spectral asymmetry, respectively. The parameter ρ is 1.44 ± 0.02 (DMPC, LysoPC, DDM) and 1.56 ± 0.02 (POPC).

Time-resolved Fluorescence Anisotropy Measurements

Protein alone (5 μ M) and nanodisc samples (3–10 μ M based on protein content) were prepared in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4 and deoxygenated on a Schlenk line by 3 sets of 3 evacuation/Ar fill cycles over 2 h. Data collection was taken at 28 °C using a thermostated cuvette holder and circulating water bath. The excitation source was the fourth harmonic (292 nm, 110–180 μ W, 1 kHz) of a regeneratively amplified femtosecond Ti:sapphire (Clark-MXR) pumped optical parametric amplifier laser (Light Conversion). A picosecond streak camera (Hamamatsu C5680) in photon counting mode was used for detection of Trp emission ($\lambda_{obs} = 325$ –400 nm, selected by short (< 400 nm) and long (> 325 nm) pass filters). Polarized Trp excited state decays were measured simultaneously with an optical fiber array and corrected for differences in collection efficiency of vertically and horizontally polarized light using the Trp model complex, N-acetyl-tryptophanamide (NATA). A minimum of 10,000 counts were collected for highest channel for each polarization component. Steady-state fluorescence spectra were taken before and after laser data collection to ensure samples received minimal photodamage. Fluorescence anisotropy decays were calculated according eq 4.

$$r(t) = \frac{I_{\parallel}(t) - \alpha(t) I_{\perp}(t)}{I_{\parallel}(t) + 2\alpha(t) I_{\perp}(t)} \quad (4)$$

where $r(t)$ is the apparent anisotropy, $I_{\parallel}(t)$ is the vertical polarization component, $I_{\perp}(t)$ is the horizontal polarization and $\alpha(t)$ is the ratio $I_{\parallel}(t) / I_{\perp}(t)$ for NATA. Anisotropy data were logarithmically compressed (100 points per decade) using a custom MATLAB program (Jay Winkler, Caltech). Fluorescence lifetime data were fit using IGOR Pro 6.22A to three exponential decay functions (W42_{buffer}: 0.5, 1.8, and 3.6 ns, $\chi^2 = 0.7$; W54_{buffer}: 0.6, 2.0, and 4.2 ns, $\chi^2 = 0.8$; W65_{buffer}: 0.6, 2.0, and 3.9 ns, $\chi^2 = 0.8$; W42_{disc}: 0.6, 2.0, and 6.1 ns, $\chi^2 = 1.1$; W54_{disc}: 0.9, 2.3, and 4.7 ns, $\chi^2 = 1.1$; W65_{disc}: 0.4, 1.7, and 6.5 ns, $\chi^2 = 1.0$). Anisotropy data were globally fit using IGOR Global fit analysis. Decays for buffer were adequately fit to a single exponential ($\tau_c = 2.2$ ns) while the nanodiscs were best fit using a double exponential ($\tau_{c1} = 1.1$ ns, $\tau_{c2} = 16$ ns) while the nanodiscs were fit using a double exponential ($\tau_{c1} = 1.1$ ns, $\tau_{c2} = 16$ ns). Reduced χ^2 values for buffer and nanodisc anisotropy fits were $\chi^2_{W42,buffer} = 0.9$, $\chi^2_{W54,buffer} = 1.2$, $\chi^2_{W65,buffer} = 1.2$, $\chi^2_{W42,disc} = 0.9$, $\chi^2_{W54,disc} = 0.6$, and $\chi^2_{W65,disc} = 1.1$. All reduced chi-squared values for buffer and nanodiscs assumed a standard error of 0.004 and 0.0025, respectively. We note that the value of τ_{c2} is not accurately determined but estimated to be no faster than 10 ns.

RESULTS AND DISCUSSION

Optical Clearance of DMPC

Multilamellar vesicles (MLVs) of DMPC produce turbid solutions that can be cleared in the presence of ApoC-III, indicating membrane remodeling and formation of smaller, optically clear nanodiscs (Figure 2A). Experiments were performed whereby a small volume (5% of total solution volume) of concentrated DMPC was added to protein solution at 28 °C. This temperature, which is above the lipid gel phase transition temperature (24 °C),³³ yielded the most consistent kinetics. Both the rate and extent of clearance increase with protein concentration (Figure 2B). Without ApoC-III (brown line), the solution (600 μM DMPC in 10 mM NaPi, 100 mM NaCl, 1 mM EDTA, pH 7.4) remains turbid with a constant optical density. The addition of ApoC-III (6 μM) reduces the measured absorbance (black line); complete clearance by 20 min is observed at 15 μM (light gray line). Assembly kinetics are best fit using biexponential curves suggesting two or more steps involved in the clearance of multilamellar DMPC vesicles. A model of nanodisc formation has been previously proposed for small unilamellar vesicles, but it is not clear how the model, which depends on protein accessibility to lipid layers to induce flattening, would be directly applicable for the multilayered and the much larger MLVs.²¹ Here, ApoC-III may be interacting with DMPC MLVs first to produce intermediate structures, such as lipid tubules,³⁴ before complete conversion into nanodiscs.

Similar experiments were performed for the single-Trp containing ApoC-III mutants (10 μM) using DMPC MLVs (200 μM) at 28 and 37 °C (Figure 2C). Clearance rates between ApoC-III mutants at 28 °C are similar to that of the wild-type (WT) protein and produce

indistinguishable particles approximately 15 nm in diameter and 5 nm thick (Figure 3). Clearance is suppressed considerably at 37 °C especially for both W54 and W65, indicating that elevated temperatures can inactivate ApoC-III remodeling. The observed thermal inactivation is reversible because, upon reducing the temperature back to 28 °C, remodeling occurs (*vide infra*). At both 28 and 37 °C, our data consistently show W42 kinetics as the most similar to WT ApoC-III followed by W54 and W65. This suggests that, of the three point mutations, ApoC-III containing W42F most significantly impacts clearance kinetics of ApoC-III whereas W65F has the smallest effect.

Temperature-dependent ApoC-III Folding and Nanodisc Formation

Deviations in clearance of DMPC MLVs at 37 °C suggest differences in protein folding behavior as a function of temperature. Specifically, inactivation of W65 at 37 °C implies a defined temperature above which remodeling does not occur because the protein cannot bind to DMPC or fold properly and thus remodel MLVs. To investigate this behavior further, we employed CD spectroscopy, which is a sensitive technique in monitoring protein secondary structural changes. As expected, no spectroscopic change is observed at 45 °C in the presence of DMPC compared to protein in buffer alone, indicating ApoC-III remains unfolded and likely, unbound (Figure 4A). In contrast, at 25 °C, where ApoC-III binds and remodels DMPC, the CD spectrum develops two negative peaks at 208 and 222 nm, characteristic for α -helical structure, where the greatest change from free, disordered ApoC-III is observed at 222 nm. By converting the CD signal into mean residue ellipticity (MRE), normalized for protein length, concentration, and solution path length, we estimate ~50% helical content, comparable to what we previously reported with anionic phospholipid vesicles.²⁷

After establishing that monitoring the CD signal at 222 nm is useful to determine the transition from a disordered to a helical state upon DMPC remodeling, we can now evaluate the differences among the ApoC-III variants. When WT, W42, W54, or W65 (10 μ M) is first incubated at 45 °C followed by the addition of DMPC (500 μ M), all the proteins exhibit similar spectroscopic signatures of a disordered protein. By slowly lowering the temperature to 25 °C (20 °C/h), helical structure begins to develop for all proteins (Figure 4B). Measureable differences are now clearly apparent among the proteins where WT and W42 exhibit a comparable onset transition temperature (t_r = 39 °C) followed by W54 (t_r = 37 °C) and W65 (t_r = 33 °C). In contrast, there are minimal changes in the CD signal when the same experiment is conducted on protein alone (Figure 4B, lower panel), reaffirming that we are observing the conformational change coupled to DMPC binding and nanodisc formation. Our data would suggest that despite the commonly used Trp-to-Phe mutations, the substitution of two of the three Trp native sites in ApoC-III, especially the W42 residue, can impact helical formation and nanodisc assembly at elevated temperatures. Based on these results, we propose that the region proximal to W42 plays a role either in nucleating helix formation or in helix elongation. Alternatively, this site could be involved in initiating lipid binding. We believe that this observation is biologically meaningful as W42 is the least variant of the three Trp residues across mammalian species (Figure S1).

Purification of Nanodiscs

The presence of unincorporated lipid or protein can affect the spectroscopic and chemical analysis of ApoC-III/DMPC nanodiscs. These components were separated from the nanodiscs using size-exclusion chromatography (Figure 5A). Nanodiscs eluted with a single peak at 11.6 ± 0.2 , 11.5 ± 0.1 , 11.3 ± 0.1 , and 11.1 ± 0.1 mL for WT, W42, W54 and W65, respectively. For comparison, free WT, W42, W54, and W65 eluted at 16.7 ± 0.1 , 16.6 ± 0.2 , 16.5 ± 0.2 , and 16.3 ± 0.1 mL. A highly monodisperse population was observed by TEM and the average nanodisc diameter was determined to be 15 ± 2 nm ($n = 387$). These measurements are identical to the unpurified samples indicating that SEC did not alter the size of the nanodiscs (Figure 5B). The mean Stokes diameter of 12 nm, measured by DLS measurements, is in good agreement with TEM and nearly indistinguishable for the ApoC-III/DMPC nanodiscs formed by the different Trp variants (Figure 5C).

FTIR and CD Characterization of Nanodiscs

To ascertain that the presence of both DMPC and ApoC-III in the purified fractions, we utilized attenuated total reflection Fourier transform infrared spectroscopy (FTIR) (Figure 5D). Serving as spectral references, ApoC-III (purple) and DMPC (brown) alone in buffer are shown. The ApoC-III spectrum exhibits the polypeptide amide I and II vibrational stretches at 1652 and 1550 cm^{-1} , respectively.³⁵ The PO_2^- asymmetric stretch at 1233 cm^{-1} , characteristic of the phospholipid head group, is observed in the DMPC spectrum. Both protein- and DMPC-derived bands are clearly present in the nanodisc spectra of WT, W42, W54, and W65. In fact, the IR spectra are nearly indistinguishable among the nanodisc samples. The amide-I band is known to convey secondary structural information; however, differences between helical and disordered conformational state is difficult to discern, as is the case here.

Thus, we turned to CD measurements to evaluate differences in α -helical structure in the purified nanodiscs prepared from WT ApoC-III and mutants (Figure 5E). Minor differences are observed both in the intensity of 208 and 222 nm and their relative intensity ratios. While the significance of the ratio between the 222 and 208 nm peaks in α -helical structure is not well understood, it could suggest that there are subtle differences in helix-helix interaction between different single-Trp variants bound in nanodiscs.³⁶ Using the MRE at 222 nm, we estimate that the relative percent helicity of WT and W42 nanodiscs ($f_{\text{helix}} \approx 51\%$) are slightly greater than in W54 and W65 ($f_{\text{helix}} \approx 47\%$ and 45% , respectively). In general, the helical content of nanodiscs follows the SEC elution volumes (WT $\sim$ W42 $>$ W54 $>$ W65) which is inversely correlated to size. Based on the CD results, we interpret that the changes in SEC elution profiles to reflect differences in the amount of unstructured, water-exposed portions of ApoC-III polypeptide chain, which would contribute to apparent increases in particle size or to non-ideal interaction behavior with the gel bed. It is important to note, however, that once the nanodiscs have formed, they have similar thermal stabilities with a comparable melting temperature of 57 – 58 $^{\circ}\text{C}$ as determined by CD spectroscopy.

Number of ApoC-III in a DMPC Nanodisc

Because there were minor differences in helical content between the single-Trp mutant and WT nanodiscs, but not in their sizes, we wanted to determine whether the amount of proteins

bound per nanodisc is different for each variant and thus could account for this observation (*i.e.* fewer ApoC-III molecules per nanodisc would yield smaller helicity). DMPC concentration was determined by a chemical assay which produces molybdenum blue proportional to its phosphate content.³² Protein concentration was determined by absorbance at 280 nm based on amino acid content ($\epsilon_{\text{WT}} = 19,480 \text{ M}^{-1} \text{ cm}^{-1}$, $\epsilon_{\text{W42}}/\epsilon_{\text{W54}}/\epsilon_{\text{W65}} = 8,480 \text{ M}^{-1} \text{ cm}^{-1}$). No significant differences in the lipid-to-protein stoichiometry were observed and the average value of 24 ± 4 DMPC per ApoC-III agrees well with previous studies on human-derived ApoC-III nanodiscs.^{17, 37} Taking this value and assuming a circular disc of 150 Å in diameter and a DMPC lipid area of 59.9 Å²,³⁸ we estimate that each nanodisc is composed of approximately 590 DMPC molecules and 25 molecules of ApoC-III. If the total area of an amphipathic helix represents the protein bound area, the edge of the disc ($\sim 17,000 \text{ Å}^2$) could, within error, adequately accommodate the binding of 25 molecules of ApoC-III, each possessing approximately 50% amphipathic helix ($\sim 24,000 \text{ Å}^2$); but, it also suggests that some interaction with the face of the nanodisc is plausible. The exact orientation of the bound helices on the lipid bilayer remains to be experimentally determined. Regardless, this rough calculation still implies a considerable fraction of each bound ApoC-III remains unstructured which are free to interact with neighboring proteins or extend into the solvent. Corroborated by our CD results, differences between these unbound regions may account for the small disparity in the elution peak profiles during purification of the nanodiscs. This is in stark contrast to nanodiscs prepared from ApoA-I which are typically composed of two, highly structured protein molecules.³⁹ ApoC-III nanodiscs, however, require interaction between dozens of interacting proteins that contain significantly unstructured regions, suggesting a much more complex model.

Site-specific ApoC-III Interactions in Nanodiscs Revealed by Trp Probes

Steady-state Trp fluorescence and time-resolved anisotropy measurements were taken of purified nanodiscs and compared to the proteins alone in buffer to reveal site-specific interactions of W42, W54, and W65 in DMPC nanodiscs (Figure 6). All Trp sites show quantum yield increases and blue shifts relative to the protein alone, indicating more hydrophobic environments and reduced sidechain mobilities in nanodiscs than in buffer (Figure 6A). Spectra were fit using a lognormal distribution to obtain the positions of peak intensity (λ_{max}). In buffer, W42 and W65 have nearly identical peak position, consistent with a water-exposed Trp sidechain ($\langle \lambda_{\text{max}} \rangle = 345 \text{ nm}$, Table 1). However, W54 is slightly blue-shifted ($\langle \lambda_{\text{max}} \rangle = 340 \text{ nm}$) suggesting it is in a more ordered water surrounding or somewhat protected from the aqueous solvent. Upon nanodisc formation, the greatest change in λ_{max} ($\Delta \lambda_{\text{max}}$) is observed for W54 ($\langle \lambda_{\text{max}} \rangle = 323 \text{ nm}$, $\Delta \lambda_{\text{max}} \approx 17 \text{ nm}$), indicating it resides in the most hydrophobic surrounding followed by W42 ($\langle \lambda_{\text{max}} \rangle = 334 \text{ nm}$, $\Delta \lambda_{\text{max}} \approx 11 \text{ nm}$) and W65 ($\langle \lambda_{\text{max}} \rangle = 337 \text{ nm}$, $\Delta \lambda_{\text{max}} \approx 8 \text{ nm}$). The sum of the spectra of W42, W54, and W65 both in buffer and nanodisc approximate the WT spectra, indicating that they accurately represent the Trp sites within WT ApoC-III.

Time-resolved fluorescence anisotropy directly measures rotational motion of the Trp sidechains. In the absence of DMPC, single-Trp ApoC-III mutants show small differences in initial anisotropy ($r_0 = 0.05\text{--}0.065$, 150-ps instrument response time) followed by rapid depolarization to zero ($\tau_c = 2.2 \text{ ns}$) (Figure 6B). In contrast, all Trp sidechains experience

slower decay from r_0 for ApoC-III bound in DMPC nanodiscs. Interestingly, similar r_0 is observed for W54 bound in nanodiscs as in buffer alone, indicating that the W54 site has similar local mobility in either free or bound-state, despite possessing the largest spectral blue shift and hence the most hydrophobic environment in a nanodisc. This finding is consistent with our earlier work on ApoC-III binding to anionic vesicles.²⁷

Fits to the time-resolved anisotropy data reveal variations in the number of Trp subpopulations between lipid-free ApoC-III and nanodiscs. ApoC-III anisotropy decays in buffer alone are reasonably described by a global single exponential fit (reduced χ^2 values reported in Materials and Methods). However, the nanodisc data require biexponential decays suggesting the existence of two correlation times, a fast ($\tau_c = 1.1$ ns, ~25%) component from local Trp sidechain mobility and a slower component no faster than 10 ns (~75%). This slower correlation time could originate either from regional protein motion on the nanodisc or represent a much even slower tumbling time of the nanodiscs, estimated to be approximately 250 ns.⁴⁰

ApoC-III Trp Environments Compared in Nanodiscs, Micelles, and Vesicles

To determine if the fluorescence signatures of the three Trp sites of ApoC-III in DMPC nanodiscs are spectroscopically distinct compared to other lipid-bound states, ApoC-III interactions with ndodecyl- β -D-maltoside (DDM) and 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPC) micelles as well as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles were examined. DMPC, POPC, and LysoPC all share the same zwitterionic phosphocholine head group with varying lipid tail structure (Figure 7A). The neutral DDM detergent was chosen to evaluate head group steric effects in micelles.

In every measured lipid environment, ApoC-III mutants experience a blue shift and an increase in integrated intensity compared to the lipid-free protein (Figure 7B). Consistently, the spectral blue shifts follow the same trend as observed for that of the nanodiscs (Figure 6A) where W54 exhibits the greatest shift followed by W42 and W65 (Table 1). This indicates that W54 resides in the most hydrophobic environment while W65 is the most solvent-exposed in all lipid-bound forms. In addition, unlike W42 and W65, which have different integrated intensity increases in the presence of different lipids, the quantum yield of W54 varies little and has the smallest integrated intensity compared to W42 and W65 when bound to lipids.

A detailed analysis of lipid-bound ApoC-III mutants is shown in Figure 7C. Derived from lognormal distribution fits of the spectra, $\lambda_{\max}$, which is positively correlated to local Trp surrounding polarity, is compared against the spectral width at half intensity, Γ , which is positively correlated to the Trp conformational heterogeneity. In Figure 7C, the reported dependence between Γ and $\lambda_{\max}$ in a model Trp compound, NATA (black line) taken in different solvents of varying polarity is also shown for comparison.²³ Data appearing above this line would signal increased conformational heterogeneity. Interestingly, with the exception of the POPC vesicles data, all sites in the nanodiscs and micelle-bound forms exhibit slightly greater conformational heterogeneity than would be expected for canonical Trp fluorescence. Our data would also suggest that Trp conformational heterogeneity in

nanodiscs and micelle-bound states are comparable for all three sites with noted exception of W54 in DMPC nanodiscs, where it is in a more homogeneous environment.

Upon closer inspection, it is revealed that W65 is the most sensitive to micelle lipid headgroup ($\lambda_{\max}(\text{DDM}) = 336 \text{ nm}$; $\lambda_{\max}(\text{LysoPC}) = 339 \text{ nm}$) followed by W42 ($\lambda_{\max}(\text{DDM}) = 333 \text{ nm}$; $\lambda_{\max}(\text{LysoPC}) = 335 \text{ nm}$). In contrast, W54 remains in similar environments regardless of the detergent ($\lambda_{\max}(\text{DDM}) = 326 \text{ nm}$, $\lambda_{\max}(\text{LysoPC}) = 325 \text{ nm}$). These data show that W54 is buried deeper into the hydrophobic interior of the micelle structure, primarily determined by lipid tail, and that the Trp environment is less heterogeneous than in W42 or W65. However, this behavior is opposed in the presence of anionic SDS micelles (Table 1), where W54 appears to be the most sensitive probe. One explanation is that W54 is inside a Lys-rich region of ApoC-III and embeds deeper into the SDS micelle in order to gain favorable electrostatic interaction with the sulfate group. Deeper insertion into the LysoPC and DDM is less likely due to either unfavorable electrostatic interaction with the phosphocholine group or steric hindrance by the maltoside head group.

In contrast to micelle-bound ApoC-III, Trp mutants bound to POPC vesicles have the most similar $\lambda_{\max}$ (W42: $< \lambda_{\max} > = 11 \text{ nm}$, W54: $< \lambda_{\max} > = 16 \text{ nm}$, W65: $< \lambda_{\max} > = 7 \text{ nm}$) and integrated intensities compared to DMPC nanodiscs. Both POPC and DMPC introduce an intermediate $\lambda_{\max}$ value between DDM and LysoPC for W42 and W65 whereas for W54 the bilayer containing structures produce a greater blue shift than the micelles. Overall, these data suggest that ApoC-III adopts a unique conformation in DMPC nanodiscs more similar to a bilayer-bound state than a micelle-bound state.

Concluding Remarks

In the present study, structural features of ApoC-III bound to DMPC nanodiscs were revealed by site-specific Trp fluorescence. Particularly, striking contrast was observed between W42 and W54. W42, sitting at the lipid/water interface, was the most critical Trp residue to facilitate helix and nanodisc formation instead of W54, which resides within the hydrocarbon core. Indeed, time-resolved anisotropy measurements showed W54 remains dynamic while W42 becomes immobilized upon nanodisc formation, which supports that W42 is located in a more folded region and thus, serving a role in stability. Based on our results, we predict that the region proximal to W42 plays a vital role in lipid-binding and producing nanodiscs. Additionally, similarity between the Trp spectral signatures of nanodisc- and bilayer-bound forms indicates that ApoC-III adopts conformations that allow potential protein binding on the face of the disc. This suggestion is contrary to contemporary generalizations of nanodisc structure dominated by the well-established double-belt models of ApoA-I, but is similar to at least one previous report.¹⁹ Different binding modes would allow ApoC-III to more easily bind to HDL by requiring different binding sites than ApoA-I and, at the same time, facilitate exchange with other lipoproteins through biological recognition of its unstructured regions. Through this work, we have a better understanding of ApoC-III binding in nanodiscs, serving as the groundwork for future studies of the complex protein-protein interactions within HDL particles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ApoC-III	apolipoprotein C-III
ApoA-I	apolipoprotein A-I
CD	circular dichroism
DDM	n-dodecyl- β -D-maltoside
DLS	dynamic light scattering
DMPC	1,2-dimyristoyl- <i>sn</i> -glycero-3-phosphocholine
EDTA	ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared
HDL	high-density lipoprotein
LysoPC	1-myristoyl-2-hydroxy- <i>sn</i> -glycero-3-phosphocholine
MRE	mean residue ellipticity
MLVs	multilamellar vesicles
POPC	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine
SDS	sodium dodecyl sulfate
SEC	size-exclusion chromatography
TBST	Tris-buffered saline and Tween 20
TEM	transmission electron microscopy
W42	W54F/W65F
W54	W42F/W65F
W65	W42F/W54F

WT wild-type

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SEAEDASLLS FMQGYMKHAT²⁰
KTAKDALSSV QESQVAQQAR⁴⁰
GWVTDFSSL KDYWSTVKDK⁶⁰
FSEFWDLDP E VRPTSAVAA⁷⁹

Figure 1.

Amino acid sequence of human ApoC-III. Locations of tryptophan mutations used in this study are underlined and colored red, green, and blue for W42, W54, and W65, respectively. In addition, basic and acidic residues are colored cyan and pink, respectively.

Figure 2.

Optical clearance of DMPC MLVs by ApoC-III. (A) Representative TEM images of DMPC MLVs (left, scale bar = 500 nm) and ApoC-III/DMPC nanodiscs (right, scale bar = 50 nm). (B) Concentration dependence of WT ApoC-III (6–20 μ M, black-to-light gray) on the optical clearance of turbid DMPC MLVs (600 μ M) solutions (10 mM NaPi, 100 mM NaCl, 1 mM EDTA, pH 7.4). Optical turbidity was monitored at 500 nm and taken at 28 °C. (C) Clearance of DMPC MLVs (200 μ M) by 10 μ M WT (black), W42 (red), W54 (green), and W65 (blue) at 28 °C (solid lines) and 37 °C (dashed lines). All data are normalized to DMPC alone (brown).

Figure 3.

TEM visualization of ApoC-III/DMPC nanodiscs prepared from 20 μ M WT (black), W42 (red), W54 (green), and W65 (blue) and DMPC (200 μ M) at 28 $^{\circ}$ C in 10 mM NaP_i , 100 mM NaCl, 1 mM EDTA, pH 7.4. All scale bars are 50 nm.

Figure 4.

Effect of temperature on ApoC-III folding during nanodisc self-assembly. (A) Mean residue ellipticity (MRE) of WT ApoC-III in the absence (open squares) and presence of DMPC (solid squares) at 45 °C (left) and 25 °C (right). The position of 222 nm is denoted by the arrow. Units for MRE ($\text{deg cm}^2 \text{dmol}^{-1}$) were omitted for clarity. (B, *upper panel*) MRE as a function of temperature for WT ApoC-III and Trp mutants (10 μM) in the presence of DMPC MLVs (500 μM). WT (black), W42 (red), W54 (green), or W65 (blue) ApoC-III was incubated at 45 °C (15 min) followed by the addition DMPC MLVs. Temperature was scanned (20 °C/h) from 45 to 25 °C as indicated by the arrow. (B, *lower panel*) CD temperature scans of WT (black), W42 (red), W54 (green), or W65 (blue) ApoC-III in buffer alone (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4).

Figure 5.

Characterization of purified WT and mutant ApoC-III/DMPC nanodiscs. (A) Nanodiscs purified using a Superdex 200 10/300 GL column (4 °C) monitored by absorbance at 280 nm. Samples were prepared by incubating WT (black), W42 (red), W54 (green), or W65 (blue) ApoC-III with DMPC MLVs at 28 °C (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) for 2 h. As labeled, the nanodisc complexes eluted from 11 – 12 mL with unbound protein eluting at 16 – 17 mL. (B) Representative TEM image of purified W42 nanodiscs. Scale bar is 50 nm. (C) Distributions of Stokes radius obtained from DLS measurements. Inset shows normalized autocorrelation curves for all measurements which are overlapped. (D) FTIR spectra of purified WT (black), W42 (red), W54 (green), and W65 (blue) ApoC-III nanodiscs compared to DMPC MLVs (brown) and WT ApoC-III (purple) alone. (E) CD spectra of purified nanodiscs (3–10 μM in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4, 25 °C). Averages of independently measured spectra ($n = 4$) are shown. Units for MRE ($\text{deg cm}^2 \text{dmol}^{-1}$) were omitted for clarity.

Figure 6.

Site-specific Trp fluorescence of WT and mutant ApoC-III in nanodiscs. (A) Normalized fluorescence spectra representative of purified WT (black), W42 (red), W54 (green), and W65 (blue) DMPC nanodiscs (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4, 28 °C). (B) Time-resolved anisotropy measurements for W42 (red), W54 (green), and W65 (blue) ApoC-III variants in buffer (left, 5 μM) and purified nanodiscs (right, 3–10 μM) at 28 °C. Averages of independently measured decays ($n = 4$) are shown. Anisotropy decays of ApoC-III proteins alone in buffer were globally fit (solid lines) to a single exponential decay ($\tau_{\text{buffer}} = 2.2$ ns) whereas the nanodiscs were globally fit to a double exponential decay ($\tau_{1\text{nanodiscs}} = 1.1$ ns and $\tau_{2\text{nanodiscs}} = 16$ ns). Reduced χ^2 values are reported in Materials and Methods. Dashed black line marks $r = 0$. Residual fit values are displayed above the anisotropy traces.

Figure 7.

Comparison of DMPC nanodiscs with micelle- and vesicle-bound ApoC-III. (A) Chemical structures of the different lipid molecules: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPC), n-dodecyl- β -D-maltoside (DDM), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). (B) Representative fluorescence spectra of W42 (red), W54 (green), and W65 (blue) in the absence (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) and in the presence of LysoPC micelles (1.5 mM), DDM micelles (1.5 mM), and POPC extruded vesicles (7 mM, average diameter = 62 nm) at 28 °C. Buffer and lipid background subtractions were applied and were normalized by protein concentration (3 – 10 μ M) where appropriate. (C) Plot of position of Trp spectral maximum ($\lambda_{\max}$) and spectral width (Γ) obtained from lognormal fits of Trp emission spectra of W42, W54, and W65 in buffer alone (closed squares), nanodiscs (circles), LysoPC micelles (triangles), DDM micelles (open squares), and POPC vesicles (diamonds). Error bars represent one standard deviation of the mean for $n = 3$ independent measurements. No error bars are shown if the standard deviations are smaller than the size of the data point itself. One standard deviation for the lognormal fit of $\lambda_{\max}$ is less than 0.6 nm

for all data points. The black line represents the reported data for N-acetyl-tryptophanamide, a model Trp compound, in solvents of various polarity.²³

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Table 1Trp fluorescence of ApoC-III in different lipid environments.^a

Lipid	W42		W54		W65	
	$\lambda_{\max}(\text{nm})$	$\Gamma(\text{nm})$	$\lambda_{\max}(\text{nm})$	$\Gamma(\text{nm})$	$\lambda_{\max}(\text{nm})$	$\Gamma(\text{nm})$
buffer	345.1 $\pm$ 0.5	60.3 $\pm$ 0.5	340.0 $\pm$ 0.4	58.2 $\pm$ 0.5	344.7 $\pm$ 0.4	60.1 $\pm$ 0.6
DMPC nanodiscs	334.3 $\pm$ 0.3	53.8 $\pm$ 1.0	323.4 $\pm$ 0.3	48.1 $\pm$ 0.6	336.9 $\pm$ 0.5	56.4 $\pm$ 0.7
LysoPC micelles	335.2 $\pm$ 0.3	54.5 $\pm$ 0.1	324.8 $\pm$ 0.3	50.2 $\pm$ 0.1	339.2 $\pm$ 0.3	56.2 $\pm$ 0.2
DDM micelles	332.8 $\pm$ 0.2	53.7 $\pm$ 0.4	325.6 $\pm$ 0.1	50.5 $\pm$ 0.1	336.4 $\pm$ 0.1	56.5 $\pm$ 0.2
SDS micelles ^b	336.3 $\pm$ 0.1	58.1 $\pm$ 1.8	319.4 $\pm$ 0.1	47.6 $\pm$ 0.3	338.9 $\pm$ 0.1	58.0 $\pm$ 0.1
POPC vesicles	333.6 $\pm$ 0.6	49.6 $\pm$ 0.5	324.1 $\pm$ 0.2	44.6 $\pm$ 0.3	337.5 $\pm$ 0.5	53.1 $\pm$ 0.8
POPC/POPA vesicles ^b	333.6 $\pm$ 0.1	51.2 $\pm$ 1.6	323.3 $\pm$ 1.0	45.7 $\pm$ 3.6	336.7 $\pm$ 0.1	54.9 $\pm$ 1.2

^aData represent mean and standard deviation of the mean of $n = 3$ measurements.^bData taken from ref. 27.