Interaction of Cytochrome c With Phospholipid Model Membranes Probed by SANS

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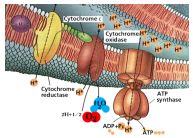
Introduction

Mitochondrial cytochrome c is a monoheme electron shuttle found between the inner and outer mitochondrial membrane and is essential for the last step in aerobic respiration delivering electrons from cytochrome c reductase to cytochrome c oxidase in eukaryotes. Interaction of mitochondrial cytochrome c with acidic phospholipids is relevant in several processes. Examples are the phospholipid-mediated association between cytochrome c and cytochrome c oxidase or activation of caspases during apoptosis by association of cytochrome c with acidic phospholipids in the mitochondrial membrane [1].

This protein-lipid association involves electrostatic and hydrophobic interactions resulting in changes in protein conformation that may affect bilayer structure as well. The primary electrostatic interaction leads to a partial unfolding of cytochrome c exposing non-polar residues to hydrocarbon chains of the lipids [2]. However, little is known about the impact on phospholipid bilayer organization.



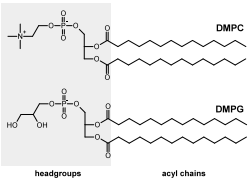
Cytochrome c in the mitochondrial membrane

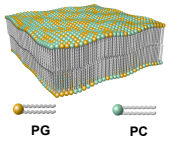
Structure model of mitochondrial cytochrome (heme group shown in yellow)

Objectives

This study investigates the effects of cytochrome c binding to a phospholipid bilayer by small angle neutron scattering (SANS). Highly monodisperse suspensions of large unilamellar vesicles consisting of zwitterionic phosphatidylcholine (PC) and negatively charged phosphatidylglycerol (PG) were used as a model membrane system to investigate the interaction with cytochrome c. The goal is to detect changes to bilayer organization and structural implications of the protein interaction with negatively charged phospholipid membranes.

Model Membrane System





Phospholipid bilayer model membranes

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-1-glycerol (DMPG) and their chain-deuterated homologs were used for the preparation of large unilamellar vesicles at DMPC/DMPG ratios of 8:2 and 6:4. At pH=7 the DMPC/DMPG mixing behavior is very close to ideal over all molar ratios [3].

Physical properties of phospholipids

V_L … molecular volume
V_H … headgroup volume
SLD_H … scattering length density of headgroup
SLD_C … scattering length density of hydrocarbon chain

lipid	Molecular formula	M_W [g/mol]	V_L [Å^3]	V_H [Å^3]	SLD_H [Å^{-2}]	SLD_C [Å^{-2}]
DMPC	$C_{36}H_{72}NO_8P$	677.94	1101	325	$1.85 \cdot 10^{-6}$	$-3.75 \cdot 10^{-7}$
DMPG	$C_{34}H_{66}O_{10}P^-$	665.86	1033	257	$2.78 \cdot 10^{-6}$	$-3.75 \cdot 10^{-7}$
D_{54}-DMPC	$C_{36}H_{18}NO_8PD_{54}$	732.26	1101	325	$1.85 \cdot 10^{-6}$	$6.87 \cdot 10^{-6}$
D_{54}-DMPG	$C_{34}H_{12}O_{10}PD_{54}^-$	720.18	1033	257	$2.78 \cdot 10^{-6}$	$6.87 \cdot 10^{-6}$

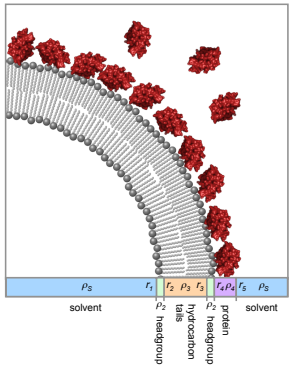
Theory

The scattered intensity from randomly distributed particles in solution can be described by

$$I(Q) = n \cdot |F(Q)|^2 \cdot S(Q)$$

Where *n* is the number of particles, *F(Q)* is the particle form factor and *S(Q)* is the interparticle correlation structure factor. For sufficiently dilute monodisperse systems *S(Q)* can be assumed equal to 1. The form factor for a centrosymmetric spherical particle *F(Q)* is the one-dimensional integral of all SLDs over all distances from the center.

$$F(Q) = -4\pi \int_0^\infty r^2 \rho(r) \frac{\sin(Qr)}{Qr} dr$$



The form factor of a unilamellar vesicle can be described by a multishell model accounting for the radial distribution of scattering length densities (SLDs). The parameters required are the diameter and size distribution of the vesicles, thickness of headgroup and hydrocarbon chain layer and the SLDs for solvent, the two headgroup regions (inner and outer leaflet) and the hydrocarbon chains.

The SLDs calculated from atomic composition and molecular volumes need to be adjusted to account for hydration. The SLD for the core equals that of the suspending solvent. This vesicle model can be extended to include additional shells to describe the protein interaction layer.

F(Q) for a spherical particle with multiple shells can be obtained by integration over the dimensions and different shell contrasts of the particle (shown here for a core with 2 layers):

$$F(Q) = 4\pi \int_0^{r_1} r^2 (\rho_1 - \rho_S) \frac{\sin(Qr)}{Qr} + 4\pi \int_{r_1}^{r_2} r^2 (\rho_2 - \rho_S) \frac{\sin(Qr)}{Qr} + 4\pi \int_{r_2}^{r_3} r^2 (\rho_3 - \rho_S) \frac{\sin(Qr)}{Qr} + \ldots$$

r_1 is the radius of the solvent core and $r_{2\ldots n}$ are the radii and $\rho_{1\ldots n}$ the SLD contrasts of the respective shells. For unilamellar vesicles the ρ_1 is equal to ρ_S.

The values for headgroup and hydrophobic layer thickness and SLDs were obtained by parameter fitting and calculation the theoretical I(Q) of the multishell model and to the experimental data. This multishell model assumes that the neutron scattering length density is homogenous within each shell.

Data Modeling

Extruded unilamellar vesicles are not uniform in size while the thickness of membrane layers remains constant. This size polydispersity can be described by a Schulz distribution applied to the particle radius where <*r*> is the average particle radius with its standard deviation σ [4].

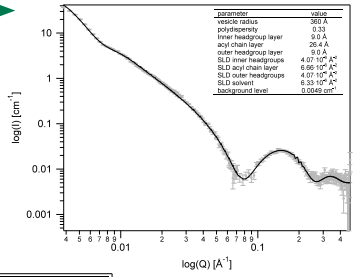
$$G(r) = \left(\frac{t+1}{\langle r \rangle}\right)^{t+1} \cdot \frac{r^t}{\Gamma(t+1)} \cdot \exp\left(-\frac{t+1}{\langle r \rangle} r\right) \qquad t = \left(\frac{\langle r \rangle}{\sigma}\right)^2 - 1$$

The calculated intensities were smeared with the distribution function assuming an average core radius and a polydispersity of ~0.3, which is typical of extruded vesicles in experiments.

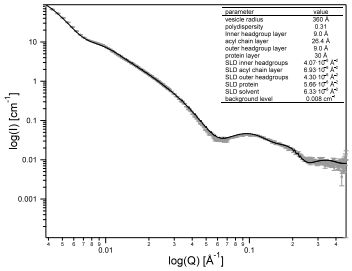
Results

Scattering profile for unilamellar D_{54}-DMPC/D_{54}-DMPG (8:2 molar ratio) vesicles in 100% D_2O. The black line corresponds to a model-dependent fit using a three shell model. Fitting parameters are listed in the table.

The estimated solvent content in the headgroup layer based on the calculated SLD for the headgroups and solvent SLD is 47%.



parameter	value
vesicle radius	360 Å
polydispersity	0.33
inner headgroup layer	9.0 Å
acyl chain layer	26.4 Å
outer headgroup layer	9.0 Å
SLD inner headgroups	4.07·10^{-6} Å^{-2}
SLD acyl chain layer	6.66·10^{-6} Å^{-2}
SLD outer headgroups	4.07·10^{-6} Å^{-2}
SLD solvent	6.33·10^{-6} Å^{-2}
background level	0.0049 cm^{-1}



parameter	value
vesicle radius	360 Å
polydispersity	0.31
inner headgroup layer	9.0 Å
acyl chain layer	26.4 Å
outer headgroup layer	9.0 Å
protein layer	30 Å
SLD inner headgroups	4.07·10^{-6} Å^{-2}
SLD acyl chain layer	6.93·10^{-6} Å^{-2}
SLD outer headgroups	4.30·10^{-6} Å^{-2}
SLD protein	5.66·10^{-6} Å^{-2}
SLD solvent	6.33·10^{-6} Å^{-2}
background level	0.008 cm^{-1}

Scattering profile for unilamellar D_{54}-DMPC/D_{54}-DMPG (8:2 molar ratio) vesicles in 100% D_2O after addition of cytochrome c. The black line corresponds to a model-dependent fit using a four shell model.

The 4th shell represents a well defined layer of cytochrome c adsorbed to the vesicle surface with a thickness of 30Å and high solvent penetration.

The model dependent fits indicate that binding of cytochrome c results in a slight increase in the SLD of the outer headgroup layer by ~6% and and increase in the SLD of the hydrocarbon chain layer by ~4%. This may indicate that the binding of cytochrome c may cause a redistribution of lipids in the bilayer resulting in a higher lipid packing density in the outer bilayer leaflet.

Conclusion

Neutron scattering was applied to study the interaction of mitochondrial cytochrome c with a phospholipid vesicle membrane bilayer model system mimicking the mitochondrial membrane. By the use of deuterated phospholipids, structural changes in the membrane bilayer can be revealed. Cytochrome c binds to a model membrane containing a mixture of neutral and negatively charged phospolipids and forms a monomolecular layer on the bilayer surface. Model dependent fits indicate that cytochrome c interacts with the phospholipid headgroup region of the bilayer and may lead to a redistribution of phospholipids affecting the packing density in the outer bilayer leaflet.

References

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