Experimental report

11/11/2015

Proposal: 8-02-703 **Council:** 4/2014

Title: Role of omega-3 fatty lipids on the interaction of Alzheimer's Ab peptide with lipid bilayers.

Research area: Soft condensed matter

This proposal is a continuation of 8-02-668

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Samples: Cholesterol (C27H46O)
POPC (C44H84NO8P)
Sphingomyelin (C41H83N2O6P)
Ab peptide (GSNKGAIIGLM)
22:6(cis)PC omega-3 fatty lipid

Instrument	Requested days	Allocated days	From	To
FIGARO	3	2	18/12/2014	20/12/2014
D17	3	0		

Abstract:

Polyunsaturated omega-3 fatty acids are increasingly proposed as dietary supplements able to reduce the risk of development or progression of the Alzheimer’s disease (AD). AD is characterized by cognitive impairment, intracellular neurofibrillary tangles, synaptic loss, and extracellular amyloid plaques. Many evidences have suggested a direct implication of lipid membranes in the mechanisms of fibrillization, favoring the Ab misfolding and aggregation. The 22:6(cis)PC is the most abundant polyunsaturated acyl chain present in the phospholipid constituting mammalian brain. Some studies suggest that fatty acids may delay or prevent the progression of AD. To date, the molecular mechanism through which these lipids act has not been yet univocally identified. In this contest, we plan to study on the effect of the 22:6(cis)PC presence in lipid bilayers mimicking the natural membrane by means of NR measurements. The aim of our research is to investigate the influence of the omega-3 lipid, 22:6(cis)PC, on the biophysical properties of lipid bilayers, and on their interaction with the amyloid peptide fragment Aβ(25−35).The role of cholesterol and sphyngomyelin presence will be also studied.

Experimental Report (N. 8-02-703)

Role of omega-3 fatty lipids on the interaction of Alzheimer's Aβ peptide with lipid bilayers.

Introduction

Omega-3 fatty acids are polyunsaturated phospholipids which are considered essential for the normal metabolism. The 22:6(cis)PC is the most abundant polyunsaturated acyl chain present in the phospholipid constituting mammalian brain. Some studies suggest that fatty acids may delay or prevent the progression of certain neurodegenerative disorders, like the Alzheimer's disease (AD). Clinical and epidemiological studies have shown that omega-3 fatty acids can affect the risk of the development of AD. AD is characterized by cognitive impairment, intracellular neurofibrillary tangles, synaptic loss, and extracellular β-amyloid plaques. Many evidences have suggested a direct implication of lipid membranes in the mechanisms of fibrillization, favoring the Aβ misfolding and aggregation.[1]

In these NR experiments, we have preliminarily investigated the effect of different amounts of 2:6(cis)PC omega-3 fatty lipid on palmitoyl-oleoyl-phosphatidylcholine (POPC) and POPC/Chol bilayers, mimicking the natural membranes. The role of 22:6(cis)PC results fundamental in modulating the Aβ peptide-membrane interaction.[2,3]

Neutron Reflectivity measurements

NR measurements have been performed on D17 reflectometer, using D_2O, 4MW and H_2O as solvent contrasts. Supported Single Lipid Bilayers (SSLBs) were prepared by vesicles adsorption on silicon bares. Small Unilamellar Vesicles (SUVs), of 25-35 nm in diameter, were formed by vortexing and sonicating for 3×10 min the MLVs suspension. The SUVs suspension (0.5 mg mL^{-1}) was injected into the NR cell, allowed to diffuse and adsorb to

the silicon surfaces over a period of 30 min. Afterward the sample cell was rinsed once with deuterated water to remove excess lipid. First, bilayers composed by only of POPC and POPC/Chol in the absence of 22:6(cis)PC were characterized. Then, bilayers composed by POPC/22:6(cis)PC and POPC/Chol/22:6(cis)PC at two composition were also studied to discriminate the effects of 22:6(cis)PC presence on the structural organization of the biomembranes. The experimental data and the best fitting curves are shown in Figure 1.

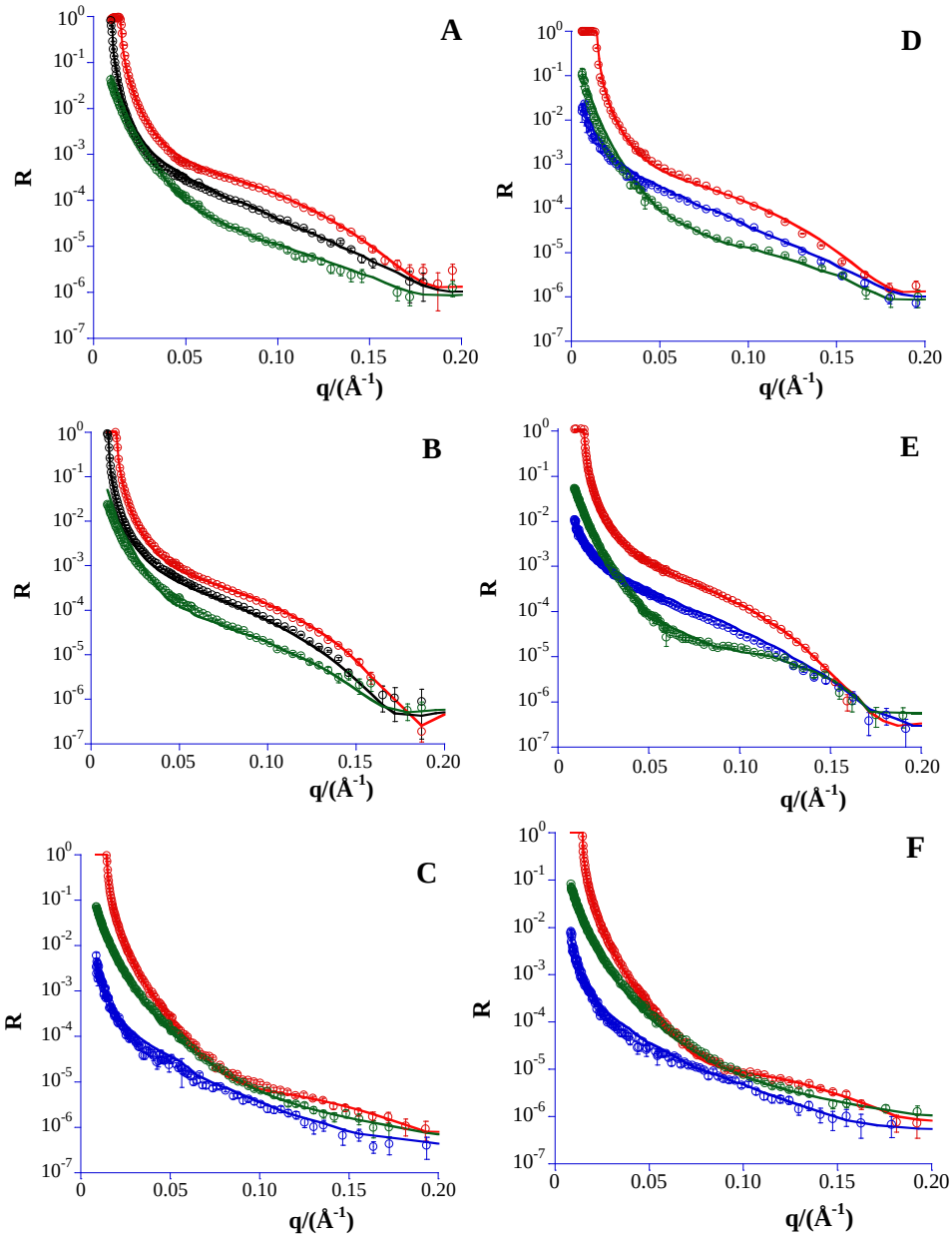

Figure 1 - NR profiles (points) and best fits (continuous lines) corresponding to the lipid bilayers formed by (**A**) POPC, (**B**) POPC/22:6(cis)PC at 75/25, (**C**) POPC/22:6(cis)PC at 25/75, (**D**) POPC/Chol at 75/25, (**E**) **(**POPC+Chol)/22:6(cis)PC at 75/25 and (**F**) **(**POPC+Chol)/22:6(cis)PC at 25/75.

In POPC membranes with a content of (22:6)cisPC equal to 25% molar fraction, a slight decrease of thickness (~2Å) and a slight increase of roughness were observed. No particular changes were also detected in the scattering length density values of hydrophobic and hydrophilic regions. Differently, at 75% molar fraction of (22:6)cisPC, a strong decrease (~16 Å) of the bilayer thickness, particularly involving the hydrophobic layer, was observed. A significant increase in the roughness values of both hydrophilic and hydrophobic regions was also observed, and a major solvent volume % was present in the hydrophilic regions..

At the same time, changes in the lipid organization of POPC/Chol were caused by the presence of (22:6)cisPC. As observed for POPC bilayers, the hightest amount of (22:6)cisPC causes deep changes in the bilayer structure. A strong decrease (~17 Å) in the total thickness value, particularly involving the hydrophobic layer, was observed. Also, a significant increase in the roughness values of both hydrophilic and hydrophobic regions was also observed, and an high solvent volume content was found in the hydrophilic regions, confirming that a great amount of (22:6)cisPC effectively modified the structural organization and lipid packing also in the case of POPC/Chol biomembranes.

Conclusions

The NR results indicates that the presence of (22:6)cisPC omega-3 lipid strongly influence the microstructure of POPC and POPC/Chol biomembranes. These changes could be influence the interaction with the Aβ peptides that we hope to investigate by NR experiments in future.

References

1. Hooijmans C.R., Pasker-de Jongb P.C.M., de Vriesa R.B.M., Ritskes-Hoitinga M. *J. Alzheim. Dis.* 28 (2012) 191–209.
2. D'Errico G., Vitiello G., Ortona O., Tedeschi A., Ramunno A., D'Ursi A. M. *Biochim. Biophys. Acta* Acta 1778 (2008) 2710–271.
3. Vitiello G., Di Marino S., D'Ursi A.M., D'Errico G. Langmuir 29 (2013) 14239−14245.