Supporting Information

Pep-Lipid Cubosomes and Vesicles Compartmentalized by Micelles from Self-Assembly of Multiple Neuroprotective Building Blocks Including a Large Peptide Hormone PACAP-DHA

Angelina Angelova,* Markus Drechsler, Vasil M. Garamus, Borislav Angelov

Abstract:

Structural control over design and formation of self-assembled nanomaterials for neuroprotection and neuroregeneration is crucial for their application in nanomedicine. Here a synthetic construct of the pituitary adenylate cyclase-activating polypeptide (PACAP38) coupled to a docosahexaenoic acid (DHA: an ω-3 polyunsaturated fatty acid (PUFA)) is designed towards the creation of compartmentalized liquid crystalline assemblies of neuroprotective compounds. The hormone PACAP38 is a ligand of the class B PAC1 G-protein-coupled receptor (GPCR), whereas DHA is a lipid trophic factor. The lipidated peptide PACAP-DHA is co-assembled into hierarchical nanostructures elaborated from hybrid vesicle-micelle reservoirs as well into PEGylated cubosomes composed of multiple neuroprotective building blocks. The resulting nanostructures are determined by synchrotron small-angle X-ray scattering (BioSAXS) and cryogenic transmission electron microscopy (cryo-TEM). Multicompartment topologies are obtained in a two-fold approach: (i) intriguing compartmentalized vesicles, which embed pep-lipid micelles forming nanopatterns, and (ii) multidomain pep-lipid cubosomes. Both kinds of topologies are favorable for sustained-release applications in combination therapies of neurodegeneration. The organizational complexity of the scaffolds involving the lipidated high-molecular weight peptide hormone is beyond the one that has been reached with small lipid-like peptide surfactants.

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1. Supplementary content about the choice of the neuroprotective compounds DHA and PACAP in relation to their important biological functions

Deficiency of docosahexaenoic acid (DHA) leads to severe pathologies because this ω-3 polyunsaturated fatty acid compound is essential for the maintenance of the retinal, neuronal and cardiovascular functions as well as for the brain development.[1-11] DHA is a lipid trophic factor, which may activate signaling pathways analogous to those triggered by peptidic trophic factors.[3,4] The anti-apoptotic effect of DHA through the ERK/MAPK signaling pathway has been associated with the regulation of Bcl-2 and Bax protein expression, the preservation of the mitochondrial membrane potential, and the inhibition of caspase activation.[11]

Regarding the therapeutic benefits of DHA, biochemical evidence has established the DHA-mediated inhibition of Aβ-amyloid fibril deposits in brain.[7-9] Using multiple mechanisms, DHA may modulate the degradation pathways and the clearance of α-synuclein in degenerated neurons. These facts are of chief significance for prospective anti-AD and anti-PD therapies.[1,2,6]

The molecular mechanism, by which DHA exerts its biological activity, involves its influence on the eicosanoid signaling pathways, regulation of genes expression in obesity and inflammatory responses, ligand stimulation of oligomeric intracellular signaling complexes (“signalosomes”), inflammatory cell signaling and Toll-like receptor signaling complexes, cellular stress responses and modifications of the lipid membranes organization.[11-25] In addition, C22:6 polyunsaturated lipids (PUFA) constitute an important fraction of the retinal cell membranes, which are responsible for vision.[11] Among other biological functions, DHA is a ligand of the retinoid X receptor.[10] The fact that DHA is a ligand of the retinoid X receptor in brain has received considerable attention in the therapeutic strategies requiring ligand activation of transcription factor proteins.[10]

In living cells, PUFA regulate the dynamic oligomerization of transmembrane receptor proteins and the membrane domain stability.[12-25] In particular, DHA influences the oligomerization kinetics of the adenosine A2A and the dopamine D2 receptors by increasing the lateral diffusion rates in the lipid bilayers.[12] Moreover, this PUFA lipid modifies the in-plane organization of cell surface-expressed membrane proteins such as the major histocompatibility complex (MHC) class I.[16]

On the other hand, the neuropeptide PACAP (pituitary adenylate cyclase-activating polypeptide) has been shown to attenuate the Aβ-amyloid (1–42)-induced toxicity.[26-30] Neurotransmitter, neuromodulator, and endocrine-paracrine regulation are representative biological functions of PACAP in various cell types.[27-48] The

pleiotropic activities of this peptide involve its effects on the adrenal gland and the cardiovascular, respiratory, and immune systems.[26,27] The PAC1 membrane receptor of PACAP belongs to the class B G-protein-coupled receptors (GPCR) and plays an essential role in neuronal survival.[41-48] The mechanism of receptor activation by the large PACAP ligand presents strong scientific interest.[32-35] A two-domain model has been proposed, in which the central and C-terminal helical segments of the polypeptide bind to the N-terminal domain of the membrane receptor PAC1.[35] The interaction of the disordered N-terminal region of PACAP with the membrane receptor stimulates the PAC1 intracellular signaling. The importance of the helical conformation of PACAP for the ligand binding to the PAC1 receptor protein has been suggested.[35]

As a 38-amino acid peptide, PACAP stimulates the cAMP formation in pituitary cells through its hormone activity.[36] PACAP has been reported to increase the levels of the anti-apoptotic proteins p-Akt, p-ERK1, p-ERK2, PKC, and Bcl-2.[28] This leads to diminished levels of activated caspases and in a decreased phorphorylation of the pro-apoptotic protein p38MAPK.[28] Therefore, this peptide appears to be a neurotrophic factor.[36-39,42,44] It is protective in retinal pathologies and contributes to retinal regeneration by attenuating the apoptosis of the retinal neurons.[27]

Both DHA and PACAP may cross the blood-brain barrier (BBB) to exert their effects. However, they are especially deficient under pathological and stress conditions.

Because of the deficiency of PACAP and DHA under neurodegenerative conditions, these compounds need to be delivered by suitable safe carriers to the central nervous system (CNS) towards an improved therapeutic outcome.

2. Supporting figures

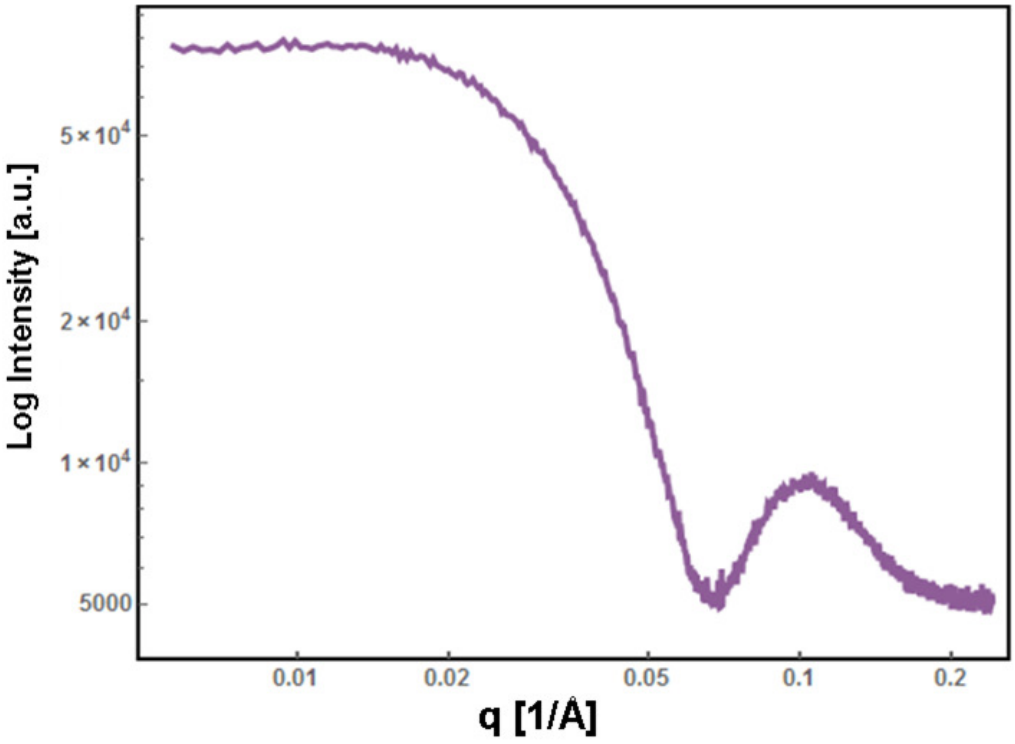

Figure S1. Synchrotron small-angle X-ray scattering (SAXS) pattern of a VPGS-PEG_{1000} micellar solution (3 mM concentration) at room temperature.

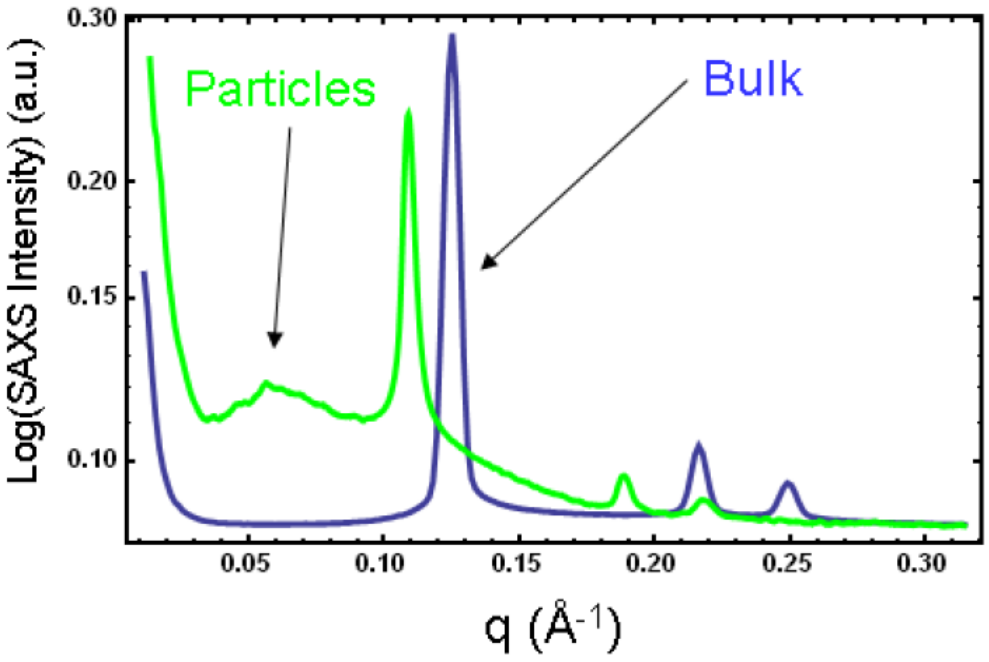

Figure S2. SAXS patterns of self-assembled bulk MO/Vitamin E and dispersed MO/Vitamin E/VPGS-PEG_{1000} mixtures showing the formation of an inverted hexagonal liquid crystalline lipid phase and hexosome particles at room temperature.

3. Supporting References

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