Experimental report

25/01/2021

Proposal: 9-13-858 **Council:** 4/2019

Title: Investigating structural organization of cell-penetrating peptides and insulin complexes

Research area: Other...

This proposal is a new proposal

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Samples: Insulin
octenyl succinic anhydride-modified hyaluronic acid
penetratin (RQIKIWFQNRRMKWKK-NH2)

Instrument	Requested days	Allocated days	From	To
D11	4	2	17/02/2020	19/02/2020

Abstract:

In this proposal we want to characterize the physico-chemical properties of self-assembling complexes of cell-penetrating peptides (CPPs) and a therapeutically relevant protein. We have assessed the efficacy of L- and D- form CPPs as potential carriers for intestinal insulin delivery, but we still lack fundamental understanding on the structure of the complexes formed between the two components upon mixing and the effect of this structure on the intestinal delivery efficacy. Scientific literature regarding structural organization of CPP-cargo complexes is currently limited to proposed models for covalent and non-covalent binding of CPPs to insulin or drug delivery systems, emphasizing the need for further characterization of such complexes. By obtaining such insight on structural organization, we will be able to interpret results of efficacy studies to a much higher extent, and thus to tailor the design of intestinal drug delivery components for optimal delivery.

Abstract

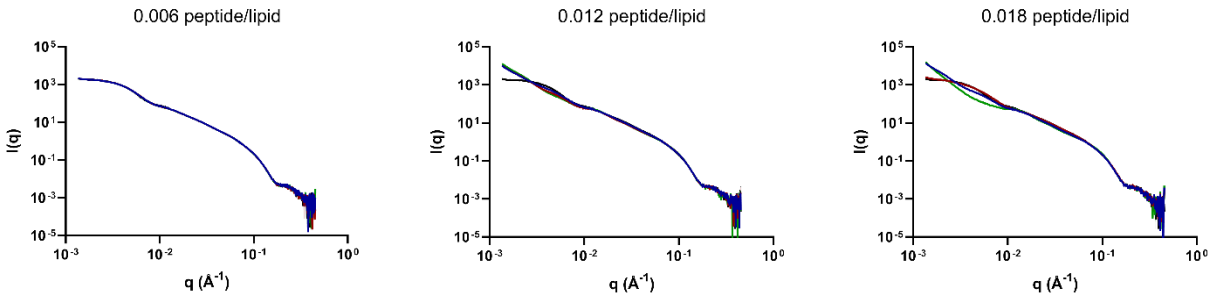
Carrier peptide-interactions with lipid bilayers were investigated for mechanistic insight on carrier-mediated insulin delivery. The scattering intensity of carrier peptide-liposomes interactions indicate a mechanism involving electrostatic interactions of the peptide with the cell membrane.

Experimental report

Poor permeation of therapeutic peptides and proteins such as insulin across the intestinal epithelium can be enhanced by cell-penetrating peptides (CPPs) applied as carriers. The mechanism of the CPPs is proposed to involve interactions between the positively charged peptides and the cell membrane, with its negatively charged phospholipids.

Carrier peptide-interactions with bilayers in the form of liposomes consisting of zwitterionic POPC and anionic POPG lipids were investigated using small-angle neutron scattering (SANS) for mechanistic insight. The liposomes alone and in presence of a small amount of peptide showed a scattering pattern characteristic for spherical vesicles (Fig. 1). The liposomes alone were fitted to a vesicle model with a radius and thickness of 280 and 36.5 Å, respectively. An increase in the scattering intensity was shown at low q (slope of 2.4-2.6) with 0.012 peptide/lipids, indicative of liposome aggregation. Interestingly, at higher peptide concentrations, the scattering reversed to the characteristic pattern for vesicles with some smearing at mid q for two of the peptides, whereas the interactions were irreversible for the third peptide.

The scattering intensity of carrier peptide-liposomes interactions together with carrier-mediated insulin delivery (data not shown) indicate a mechanism involving electrostatic interactions of the peptide with the cell membrane.



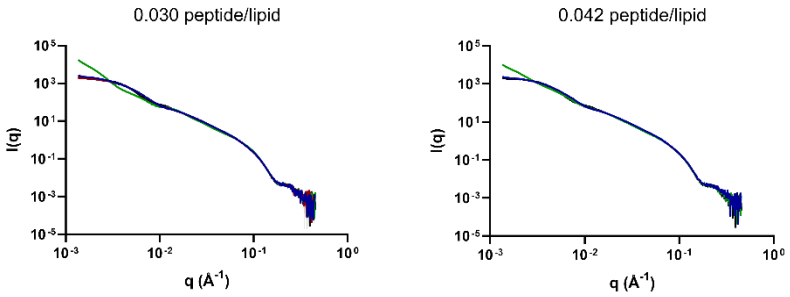


Fig. 1. Scattering intensity of liposomes consisting of 10 mg/mL 80:20% (mol/mol) POPC:POPG lipids (black) in presence of 0.18-1.24 mg/mL of three peptides (red, blue or green).

Some of the time was also used to perform additional experiments needed as prelimiary data for later SANS beamtimes. Specifically, data for two projects was obtained:

1) Self-assembly of protein superstructures

In this study, we wanted to conduct a pilot experiment to follow the size and shape change of a few different protein aggregates in different salt solution. However further investigation of the morphology of the aggregates structures is needed and ongoing information from atomic force microcopy is being obtained before further analysis of the SANS data.

2) Investigating structural organization of hyaluronic acid-based nanogels encapsulating cationic amphiphilic peptides

We investigated the internal organization of oleyl-hyaluronate nanogels loaded with one of three peptides to better understand how the peptide loading affects subsequent release from the carrier. We observed a distinct change in particle shape from a gel network to organized spherical particles upon loading of particles with the peptides (Fig.2). Some tailing at very low Q indicating aggregation of our particles was observed, which was in line with the TEM images we obtained for those samples, which shows clustering of individual particles. Additionally, the mid-Q range showed a slope of -4, indicating spherical shape of the nanogels. Fittings of the available data at higher Q suggests presence of presence of phase-separated cylindrical micelles, as indicated by a slope of -1, within the sphere-like particles. Interestingly, a change in internal structure was observed when the ratio of peptide to polymer was increased from 0.3 to 0.5, with a feature of approx. 48 Å diameter present (Figure 2B). Additional SANS experiments will be performed to further evaluate this structure.

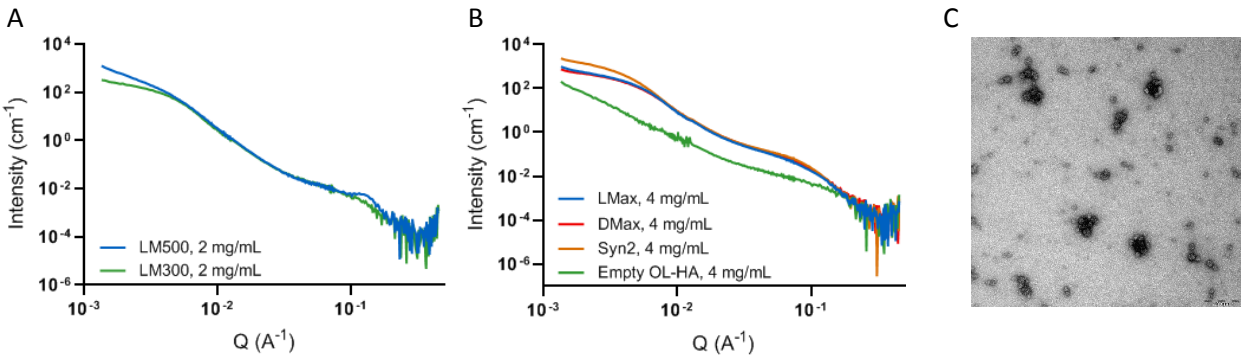


Fig. 2. Scattering intensity of nanogels composed of oleyl-hyaluronate A) obtained at various ratios of peptide-to-polymer, and B) unloaded and loaded with three peptides: Lmax, Dmax or Syn2. C) TEM micrographs of Dmax-loaded nanogels show small spherical particles and particle clustering.