Study of Sterically Stabilized Phospholipid Simple and Mixed Micelles as Nanocarriers for Peptide Drugs

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Statement of Purpose: Sterically stabilized phospholipid micelles (SSM) and mixed micelles (SSMM), composed of PEGylated lipids and PEGylated lipids plus phosphatidylcholine (PC) respectively, are two promising lipid-based delivery systems that have been studied extensively in our laboratory[1,2]. They can be used as nanocarriers for water-insoluble inorganic molecules and peptide drugs. Previously, we have shown that association of peptides with SSM increased stability as well as bioactivity of peptide molecules[1]. However, the association of peptide molecules with SSMM has not been studied.

It is most likely that peptide molecules interact with SSM at the micellar polyethylene glycol (PEG) palisade region. Therefore, with the addition of PC to SSM, it is not clear if the PEG palisade of SSMM will behave similar to or better than SSM as a nanocarrier for peptides. The purpose of this study was to investigate the potential of SSMM as a nanocarrier for peptide drugs and to compare this micellar system with SSM.

Methods: SSM composed of distearoyl-phosphatidylethanolamine-polyethyleneglycol-2000 ($DSPE\text{-}PEG_{2000}$) and SSMM composed of $DSPE\text{-}PEG_{2000}$ and egg PC (molar ratio: 90:10) were prepared as previously described in our laboratory[2]. The particle sizes of SSM (15 ± 2 nm) and SSMM (14 ± 2 nm) were measured by quasi-elastic light scattering (NICOMP 380) and were not significantly different.

Three peptide drugs were used as models: vasoactive intestinal peptide (VIP), glucagon like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). To each peptide, at a fixed concentration, varying concentrations of SSM or SSMM was added (molar ratio of lipid to peptide varying from 0 to 80) followed by 2hr incubation at 25°C. The fluorescence emission spectra of samples were measured using SLM Aminco 8000 Spectrofluorimeter. For each peptide in SSM or SSMM, the fluorescence intensity enhancement (I/Io) at Em_{max} was plotted against lipid:peptide molar ratio to obtain the lipid:peptide saturation curve. The data was then fitted into a simple, rectangular hyperbola curve using SigmaPlot® to determine lipid:peptide saturation ratio. The maximum number of peptide molecules that could interact with each micelle was calculated from the aggregation number of lipid monomers per micelle (~90) for both SSM and SSMM[2].

Results/Discussion: A significant increase in fluorescence intensity was observed when peptides were incubated with SSM or SSMM (Figure 1), indicating that peptides interacted with micelles.

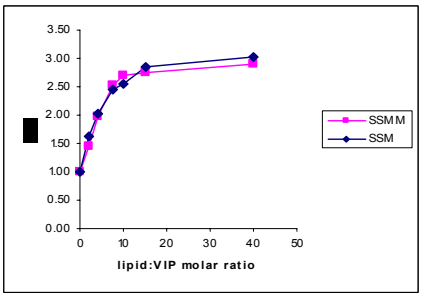

Figure 1. Lipid:VIP saturation curves in SSM and SSMM (n=3)

The lipid:peptide saturation curves of VIP, GLP-1 and GIP in association with SSM or SSMM did not differ significantly between the two micellar systems. Figure 1 shows a representative plot for VIP. Lipid:peptide saturation ratio and the maximum number of peptides that interacted with each micelle were not significantly different between SSM and SSMM for each peptide (Table1).

Peptide	# of a.a residues	Micellar system	Lipid:peptide saturation ratio	# of peptide/micelle
VIP	28	SSM	39.9 ± 7.3	2.3 ± 0.4
		SSMM	43.3 ± 3.6	2.1 ± 0.2
GLP-1	30	SSM	29.0 ± 5.2	3.2 ± 0.5
		SSMM	35.6 ± 15.1	2.9 ± 1.2
GIP	42	SSM	75.9 ± 27.6	1.3 ± 0.4
		SSMM	91.6 ±18.8	1.0 ± 0.2

Table 1. Peptide association with SSM and SSMM

The results showed that the lipid:peptide saturation ratios were not affected by the addition of PC to SSM. It is possible that the flexible PEG chains of the micellar palisade are able to undergo folding or stretching to accommodate the associating peptide molecules. This will minimize any differences between the palisade capacity of SSM and SSMM in the uptake of peptides. On the other hand, the number of peptide molecules that could interact with each micelle (SSM or SSMM) was significantly different between GIP and the other 2 peptides (Table 1). A possible reason could be the size of peptide molecules, as GIP has a longer chain length compared to the other two peptides. However, further studies are needed before any general conclusion can be made.

Conclusions: The presence of PC in SSMM did not affect the interaction of peptides with micelles for VIP, GLP-1 and GIP. The same number of peptide molecules was able to interact with each SSM or SSMM. However, peptide-micelle interaction was observed to be different for the bigger peptide (GIP) compared to GLP-1 and VIP.

References:

1. Krishnadas A, et al. Curr Pharm Des. 2003;9(12):1005-12.
2. Ashok B, et al. J Pharm Sci 2004; 93: 2476-2487.

Acknowledgements: VA merit Review and NIH grants R01 AG024026, R01 HL72323.