Do Peptide Drugs Interact with Bile Salts in the Gastrointestinal Environment?

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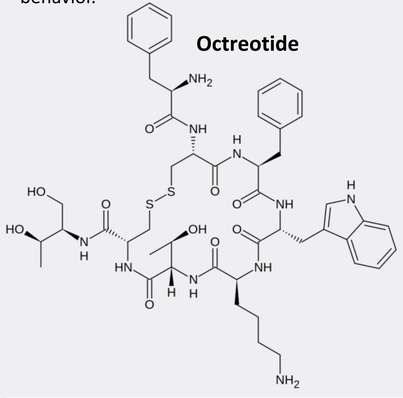
Introduction

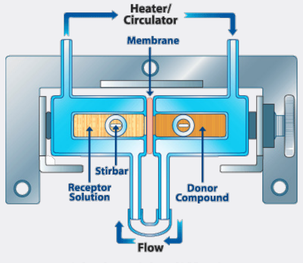
- The unique physicochemical properties of peptide drugs *e.g.* large molecular weight, low membrane permeability & susceptibility to gastrointestinal enzymatic degradation typically necessitate that they be administered via injection [1].
- Semaglutide tablets (Rybelsus®) & octreotide capsules (Mycapssa®) received FDA approval in 2019 & 2020, respectively, indicating that oral peptide pharmacotherapy is possible.
- Experiments with bile-duct cannulated rodents demonstrated that octreotide intestinal absorption was decreased 6-fold in the presence vs. absence of bile [2].
- The effect of simple bile salt micelles & bile salt/phospholipid mixed micelles present in the gastrointestinal milieu on peptide drug solution behavior has not been investigated.
- Large inter- & intra-individual variability in bile salt concentrations *in vivo* may contribute to low & variable oral peptide absorption/bioavailability.
- Bile salts are unique amphiphiles that aggregate in solution to form simple micelles. A two-step model is proposed; primary micelles form via hydrophobic interactions, then for dihydroxy bile salts, secondary micelles form via hydrogen bonding interactions between the primary micelles [3].
- Commercially available biorelevant fasted state simulated intestinal fluid (FaSSIF) & fed state simulated intestinal fluid (FeSSIF) contain taurocholate as a model bile salt.
- Our aim is to determine & understand the impact of bile salt micelles on peptide drug solution behavior.

Octreotide



Octreotide Properties	
MW (g/mol)	1019
Sequence	H_2N-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol
pKa(s)	7.00 & 10.15
Isoelectric point	12
cLogP	1 (acetate salt)
Aqueous solubility	>10 mg/mL

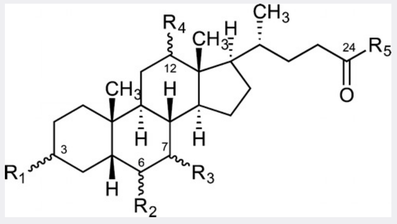
Concentration (mM)	FaSSIF (pH 6.5)	FeSSIF (pH 5.0)
Taurocholate	3	15
Phospholipid	0.75	3.75
Sodium	148	319
Chloride	106	203
Phosphate	29	
Acetic acid		144



Side-by-side diffusion cell setup

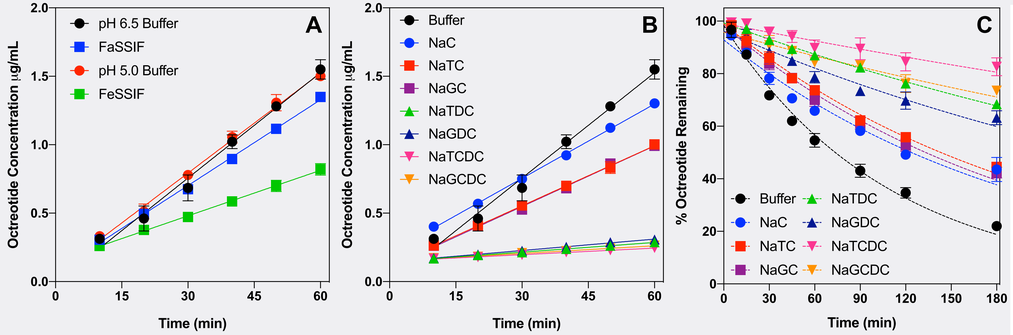
3.5 kDa regenerated cellulose dialysis membrane

Results



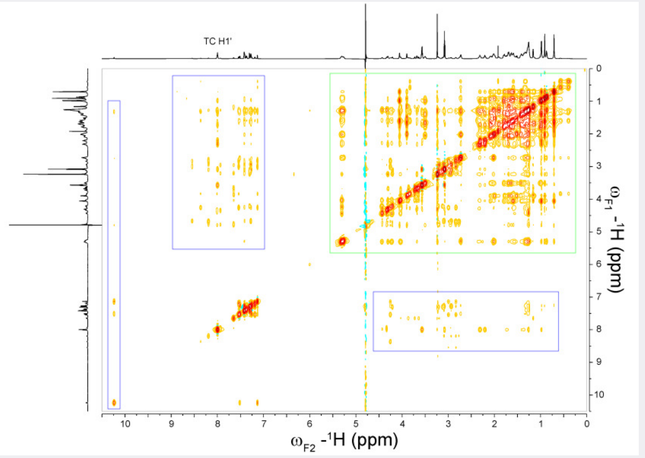
Bile Salt	R1	R2	R3	R4	R5	% in Human Bile [4]	CMC (mM)
Cholate (NaC)	OH(α)	H	OH(α)	OH(α)	O^-	trace	10.74 ± 0.09
Taurocholate (NaTC)	OH(α)	H	OH(α)	OH(α)	$NHCH_2CH_2SO_3^-$	10	9.65 ± 0.29
Glycocholate (NaGC)	OH(α)	H	OH(α)	OH(α)	$NHCH_2COO^-$	30	10.09 ± 0.37
Taurodeoxycholate (NaTDC)	OH(α)	H	H	OH(α)	$NHCH_2CH_2SO_3^-$	10	1.68 ± 0.05
Glycodeoxycholate (NaGDC)	OH(α)	H	H	OH(α)	$NHCH_2COO^-$	15	1.82 ± 0.08
Taurochenodeoxycholate (NaTCDC)	OH(α)	H	OH(α)	H	$NHCH_2CH_2SO_3^-$	5	1.82 ± 0.02
Glycochenodeoxycholate (NaGCDC)	OH(α)	H	OH(α)	H	$NHCH_2COO^-$	30	2.13 ± 0.05

- FaSSIF & FeSSIF commercial biorelevant media significantly decreased octreotide flux relative to buffer by 20 & 54%, respectively (Fig A). All 15 mM micellar bile salt solutions significantly reduced octreotide flux; dihydroxy bile salts had a much larger effect than trihydroxy bile salts (Fig B). Micellar sequestration of octreotide had a positive effect on octreotide enzymatic stability (Fig C).



Bile Salt	-log D (m^2/s)	Hydrodynamic Radius (nm)	Micelle Volume (nm^3)
NaC	9.50	0.77	1.88
NaTC	9.48	0.74	1.70
NaGC	9.45	0.69	1.38
NaTDC	9.76	1.41	11.74
NaGDC	9.73	1.32	9.55
NaTCDC	9.80	1.53	15.06
NaGCDC	9.80	1.55	15.48

- Diffusion-ordered spectroscopy (DOSY) NMR verified the different solution behavior of trihydroxy vs. dihydroxy bile salts with/without octreotide.
- Significant ^1H NMR chemical shift perturbations were observed when bile salts (0-15 mM) were titrated against 0.1 mM octreotide. Chemical shift perturbations & peak broadening for octreotide indole, amide & aromatic protons were greatest in the presence of dihydroxy bile salts.



- Nuclear Overhauser Effect Spectroscopy (NOESY) NMR revealed intermolecular NOEs of FaSSIF (green box) as well as intermolecular NOEs of octreotide & FaSSIF (blue boxes). Experiments were also performed in 15 mM NaTC, NaTDC & NaTCDC solutions. NOEs between peptide aromatic protons & bile salt methyl/steroid ring protons were observed.

Conclusions & Future Directions

- Bile salt micelles have a significant impact on octreotide membrane flux *in vitro*; dihydroxy bile salts have a larger effect than trihydroxy bile salts.
- For amphiphilic & water-soluble peptide drugs like octreotide, interaction with bile salt micelles & mixed micelles in the gastrointestinal environment may negatively affect membrane flux & oral absorption/bioavailability. Characterizing & understanding bile salt interactions is critical.
- Investigations with Ala-mutated octreotide analogs & other amphiphilic peptide drugs *e.g.* desmopressin, cyclosporine are ongoing.

References

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2. Fricker J *et al.* 1992, BJP, 105(4).
3. Coello A *et al.* 1996, J Pharm Sci, 85(9).
4. Riethorst D *et al.* 2016, J Pharm Sci, 105(2).