Structural and Dynamic Features of Plecanatide: Insights from Molecular Dynamics Simulations

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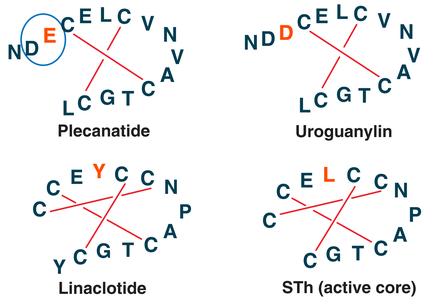
Background

- Plecanatide is the first uroguanylin analog to be tested as a therapeutic treatment of chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C)[1,2]
- Uroguanylin is an endogenous ligand that binds to the guanylate cyclase-C (GC-C) receptor in a pH-dependent manner[3]
- The GC-C receptor in the gastrointestinal (GI) tract plays an important role in the maintenance of intestinal fluid and electrolyte balance and thus has become a target for treating constipation
- At approximately pH 5, uroguanylin assumes a conformation that is most active. This pH environment is found in the duodenum and proximal jejunum
- The family of heat stable enterotoxins (ST) include STh and STp. ST peptides are stabilized by three disulfide bonds and bind to GC-C receptors with undifferentiated affinity across the range of pH environments in the GI tract, often leading to diarrhea[4]
- Linaclotide, a synthetic analog of the STh, is available for the treatment of CIC and IBS-C. Linaclotide retains the structural characteristics of STh, including the presence of three disulfide bonds and the absence of pH-sensitive amino acids on its N-terminus[5]

Aim

This study compares the structural and dynamic features of plecanatide with those of uroguanylin, linaclotide and STh across several pH values.

Peptides



Plecanatide and linaclotide are synthetic GC-C agonists and share similar amino acid sequences with natural GC-C agonists, uroguanylin and STh, respectively. In the structure of plecanatide, the aspartic acid residue found in the N-terminus of uroguanylin (D, orange) is substituted with glutamic acid (E, orange). Circle indicates pH-sensing amino acid residues of plecanatide (Asp/Glu) that were differentially protonated to reflect pH values 2, 5 & >7. Linaclotide also exhibits one amino acid substitution (orange) vs. STh. STh sequence represents the active core of the peptide.

Structural Analysis

Peptide preparation

The NMR structures of uroguanylin (PDB ID: 1UYA) and the modified crystal structure of the ST peptide (PDB ID: 1ETN) were used as starting points for the MD simulations.

Plecanatide and linaclotide were prepared by appropriately modifying the uroguanylin and ST peptide sequences, respectively, using the builder tools in molecular operating environment [(MOE) www.chemcomp.com]

Molecular Dynamics Simulations

All MD simulations of plecanatide, STh and linaclotide were performed and analyzed using the GROMACS 4.5 simulation package 26. The Amber 99 force field was used.

MD simulations of the crystal structure of STh used the structures residues, 5-17, of the full heat-stable enterotoxin (C-C-E-L-C-C-N-P-A-C-T-G-C), considered to be the pharmacophore of the molecule.

Four protonation states of plecanatide were modeled: pH 2 (Asp2, Glu3), pH 5 (Asp2-, Glu3; Asp2, Glu3-) and pH >7 (Asp2-, Glu3-). The initial structure of each peptide was placed in a cubic box with TIP 3P water and energy minimized using a Steepest Descent Minimization Algorithm. The system was equilibrated via a 50 ps MD simulation at 310 K in a NVT canonical environment followed by an additional 50 ps simulation at constant pressure of 1 atm (NPT). After the equilibration phases, a 500 ns MD simulation was performed at constant temperature (310 K) and pressure with a time step of 2 fs. The system energy and peptide spatial coordinates (trajectory file) were stored every 300 ps for further studies. All MD simulations were run in triplicate for each peptide.

After removing the first 100 ns, considered as system stabilization time, the remaining 400 ns of the MD trajectory of each group of triplicate experiments were combined using the tricat function. The combined trajectories were examined using the g-cluster function, setting gromos as clustering method with a root-mean-square-deviation (RMSD) cut-off of 0.1 nm. The different structural cluster groups were obtained as a pdb file. Cluster groups representing at least 10% of the total population for each peptide were selected as the most representative structure for that peptide.

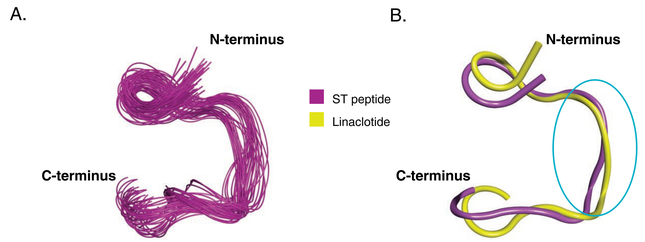
RMSD Comparisons

The representative cluster conformations of the different peptides were used for the RMSD comparison against the conformation of the STh cluster.

RMSD comparisons were performed using MOE with the major STh cluster structure serving as a reference structure for the superimposition of other peptide conformations.

Results

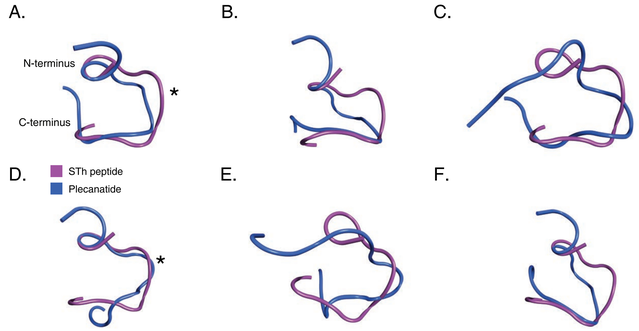
Fig 1. MD Simulations of STh and Linaclotide



A. Overlapping snapshots of STh subjected to molecular dynamics perturbations reveal that the peptide adopts a single, stable structure with little flexibility.

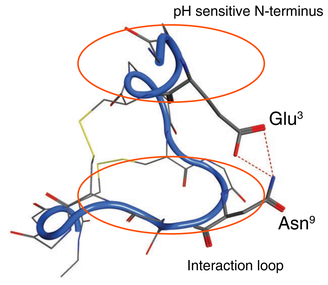
B. Superposition of representative structures from the STh (purple) and linaclotide (yellow), show that linaclotide adopts a similar conformation as STh, especially within the region of the GC-C interaction loop (circle). Variation at the C-terminal reflects changes in conformation induced by the additional tyrosine residue in linaclotide.

Fig 2. MD Simulations of Plecanatide at pH 2, 5 and >7



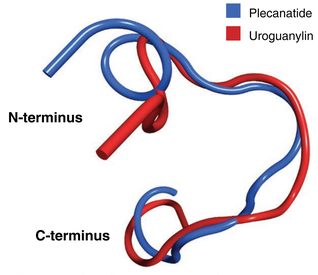
Overlapping snapshots of plecanatide (blue) and STh (purple) at pH 5 (A-D), pH 2 (E) and pH >7 (F). The overlapping conformation of the interaction loops in A and D (*) suggest that these are active forms of plecanatide.

Fig 3. Plecanatide at pH 5



At pH 5, in the Asp/Glu- ionization state, plecanatide is stabilized in an active conformation by the interaction between Glu^3 and Asn^9.

Fig 4. Most Active Conformation of Plecanatide and Uroguanylin



The most active conformation of plecanatide (Asp/Glu-) in blue and the most active NMR structure of uroguanylin in red. The interaction loop of the two peptides overlaps generally very well. Some variability is observed in the N-terminus, where the substitution Asp^2/Glu^2 is present.

Table 1. RMSD Comparison Between STa and Different Peptides Simulated

	RMSD - Å
Linaclotide	1.28
Uroguanylin	1.2 - 2.34[a]
Plecanatide - pH > 7	2.54
Plecanatide - pH 5 (Asp^- /Glu)	3.44; 2.48; 2.38[b]
Plecanatide - pH 5 (Asp/Glu^-)	1.93
Plecanatide - pH 2	3.45

[a] Range of RMSD between STh and the 10 NMR conformations of uroguanylin.

[b] Three representative clusters (> 10%) were obtained from this peptide.

Linaclotide, with a RMSD of 1.28 Å, is more similar in structure to STh. In contrast, the RMSD of the four plecanatide peptides are more similar to that of uroguanylin, with the pH 5.0 structures sharing greatest similarities to uroguanylin.

Conclusions

- **MD simulations reveal that plecanatide posesses a high degree of pH-dependent structural flexibility across the range of tested pH values. At pH 5, it assumed an active conformation**
- **Plecanatide assumed an active conformation at pH 5, where its structure can be stabilized by the interaction of Glu^3 and Asn^9**
- **Structural comparisons were quantified with RMSD values reveal that plecanatide is more similar to uroguanylin and linaclotide is more similar to STh in structure**
- **These findings suggest that plecanatide reaches maximal biological activity in the slightly acidic regions of the proximal small intestine and is less active in more basic regions - this differs from the rigid, constituitively active conformers of STh and linaclotide**

References

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Acknowledgements

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