Software assisted analysis for peptide catabolism

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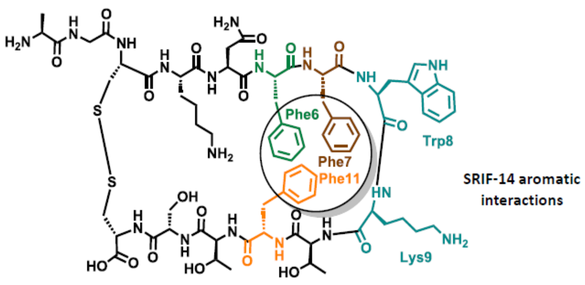
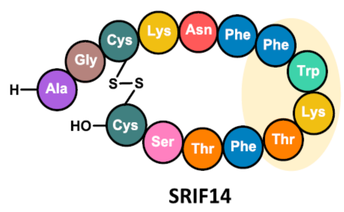
Introduction

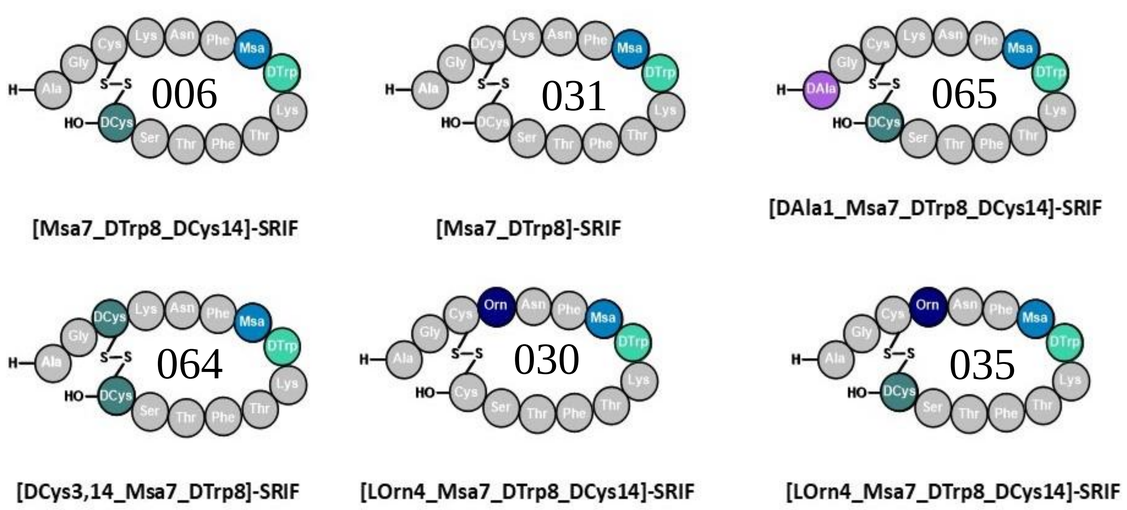
The interest in using peptide molecules as therapeutic agents is due to their high selectivity and efficacy. However, most peptide-derived drugs cannot be administered orally because of their instability in the gastrointestinal tract. To achieve better ADME properties the following chemical modifications are typically applied: substitution of the common L-amino acids to D-amino acids, cyclization of the peptide and others. These changes should improve the physicochemical and pharmacokinetics properties of the peptide. Therefore, it is crucial to evaluate these properties rapidly in early stages of drug development.

Somatostatin or Somatotropin release-inhibiting factor (SRIF14) is a natural hormone that is being used as gastric anti-secretory drug as well as to treat growth hormone secretion disorders and endocrine tumors. The substitution of phenylalanines, using non-natural aromatic amino acids to enhance the aromatic interactions, naturally present in the hormone between Phe6, Phe7 and Phe11 has been studied before[1]. Herein, we used MS data of somatostatin and six synthetic analogues to find metabolite peaks and elucidate their structures. Moreover, we could evaluate their peptide half-life to determine the effect of the applied chemical modifications.





Mass spectrometry (MS) is an essential tool for efficient and reliable quantitative and qualitative analyses: peptide-parent loss over time and metabolite formation and identification. Since the task of metabolite characterization from MS data is very time-consuming, several semi-automated tools were developed for full scan/data-dependent MS/MS peptide data interpretation (SEQUEST, etc.), but these approaches have difficulties with sequencing cyclic peptides and limited to the 20 standard amino acids. We worked to develop a new methodology to analyze peptide structure and amide bond metabolic stability based on the peptide structure (linear/cyclic, natural/unnatural amino acids). This approach uses LC-HRMS to obtain the analytical data from in vitro incubations[2].



Results

Our approach revealed not only the metabolite structures and the rates of catabolism but also the site of cleavage. We evaluated the effect of small chemical changes in the half-life of the parent compound and the structure of the firstly formed metabolite. This allowed to understand the major metabolic clearance pathway and consequently would help in the design of new analogs with improved stability.

We found that for all investigated peptides the first metabolite came from the cleavage of the linear chain Ala1-Gly2 and Gly2-Cys3 despite the fact that in compound 065 Ala1 was replaced to DAla1 (see Tables 1 and 2). Moreover, we revealed that somatostatin was cleaved at the β-hairpin area at Phe7/Phe8, Phe8/Lys9, Trp8/Lys9, Lys9/Thr10 linkages. On one hand, the substitution of the Phe7 to Msa and Trp8 to DTrp prevented cleavage in this area in compound 031. On the other hand, when these changes were combined with replacement of the Cys14 to D-Cys or Lys4 to Orn they did not stop metabolism in the β-hairpin area in Trp8-Lys9 and Lys9-Thr10 linkages as it can be seen for compounds 006, 030, 035 and 064.

Table 1. Proposed metabolite structures for somatostatin and synthetic analogues

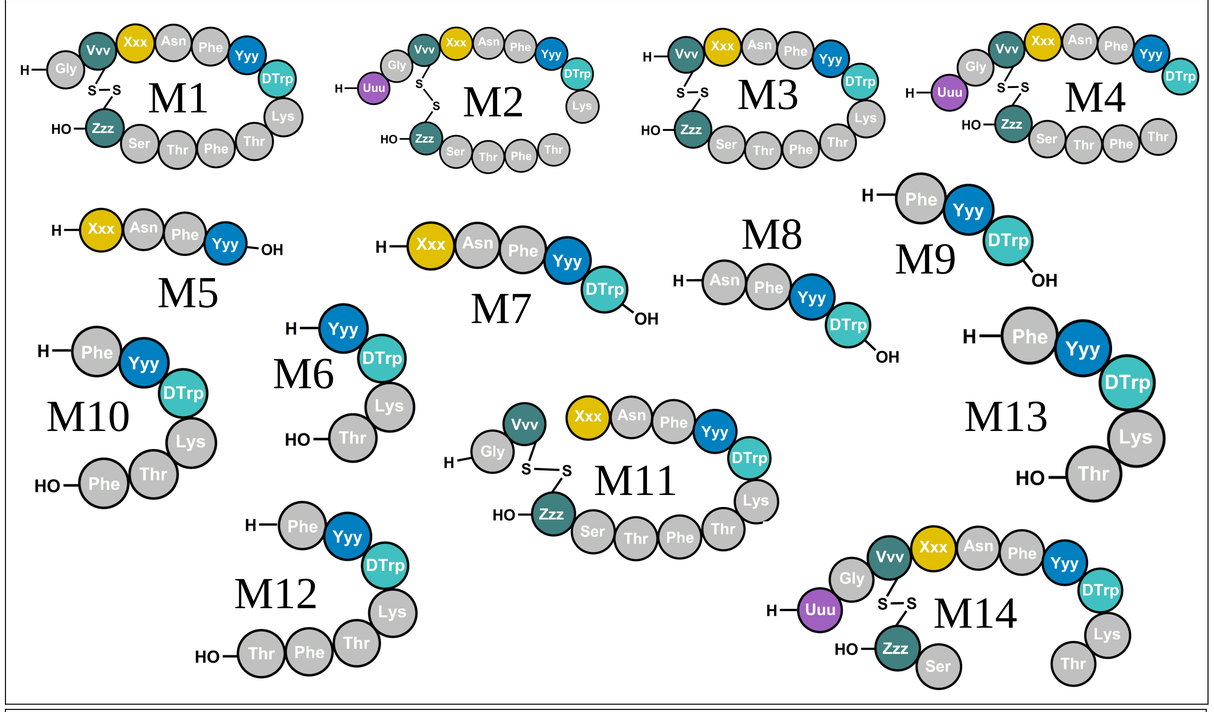


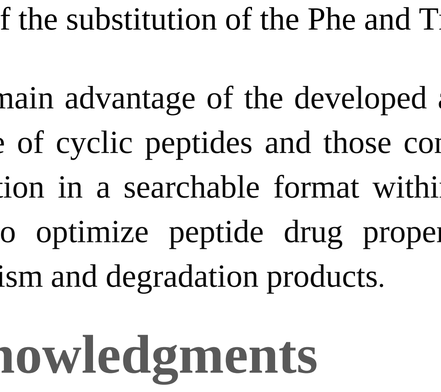
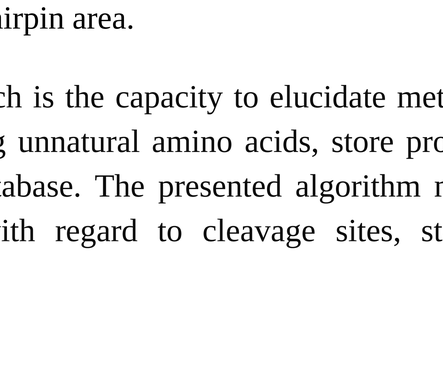
Table 2. Metabolites identified for somatostatin and synthetic analogues

Metabolites	Parent	Metabolites	Parent
M1	SRIF14, 006, 030, 031, 035, 064, 065	M8	SRIF14
M2	SRIF14, 006, 064	M9	SRIF14
M3	SRIF14, 006, 030, 035, 065	M10	065
M4	SRIF14, 006, 030, 035	M11	SRIF14, 030, 031, 035, 064
M5	SRIF14	M12	030, 031
M6	SRIF14	M13	006, 064, 065
M7	SRIF14	M14	065

Table 3. Half-life time of somatostatin analogues

Compound name	Half-life (slope)	R2 score
Somatostatin	15 min	0.993
Compound 006	30 min	0.978
Compound 030	15 min	0.993
Compound 031	27 min	0.999
Compound 035	13 min	0.999
Compound 064	65 min	0.995
Compound 065	39 min	0.953

Table 4. Metabolic stability of somatostatin and compound 064

Somatostatin	064
	

For each peptide its half-life was measured (see Tables 3 and 4). The modification of the Phe7 and Trp8 in compound 031 doubles the stability of the compound with respect to somatostatin from 15 min to 30 min. Further analysis of the effect of chemical modifications in somatostatin analogs revealed that additional substitution of Cys14 to D-Cys as in compounds 006 and 065 does not modify stability of the peptide as well as modification of Ala1. Moreover, the change of Cys3 increases half-life time of compound 064 by four times, from 15 min to 60 min. On the other hand modification of the Lys4 to Orn as in compounds 030 and 035 does not increase the half-life time of these compounds despite of the substitution of the Phe and Trp in hairpin area.

The main advantage of the developed approach is the capacity to elucidate metabolite structure of cyclic peptides and those containing unnatural amino acids, store processed information in a searchable format within a database. The presented algorithm may be useful to optimize peptide drug properties with regard to cleavage sites, stability, metabolism and degradation products.

Acknowledgments

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[2] T. Radchenko, A. Brink, Y. Siegrist, C. Kochansky, A. Bateman, F. Fontaine, L. Morettoni, I. Zamora, PLoS ONE 12(11): e0186461.