A MS approach to understand the most metabolically liable amide bonds in peptides

Ismael Zamora[2,3], Tatiana Radchenko[1], Elisabeth Ortega-Carrasco[2]
[1] Universitat Pompeu Fabra, Pl. de la Merce, 10-12, Barcelona, 08002, Spain.
[2]Lead Molecular Design, S.L., Valles St. 96-102, Local 27, Sant Cugat de Valles , 08173, Spain.
[3]Molecular Discovery, London, UK

Introduction

Peptide drugs are an important group of therapeutics. Assessment of stability *in vitro*, the identification of potential cleavage sites, structural elucidation of degradation products and also the proteases likely being involved in degradation are important information to optimize the ADME properties of these agents. Several in-silico methodologies such as PeptideCutter, PoPS, PROSPER, SitePredicting can be used to predict peptide cleavage sites for different proteases. The main limitations of these approaches is that their predictions are not expanded other the 20 natural amino acids and they experience difficulties with making predictions on cyclic peptides. The aim of this poster is to present a new approach based on mass spectrometry data to analyze peptides and their metabolite structures which could be used to predict the specific metabolic cleavage site in the examined peptide for the specific proteases.

Results

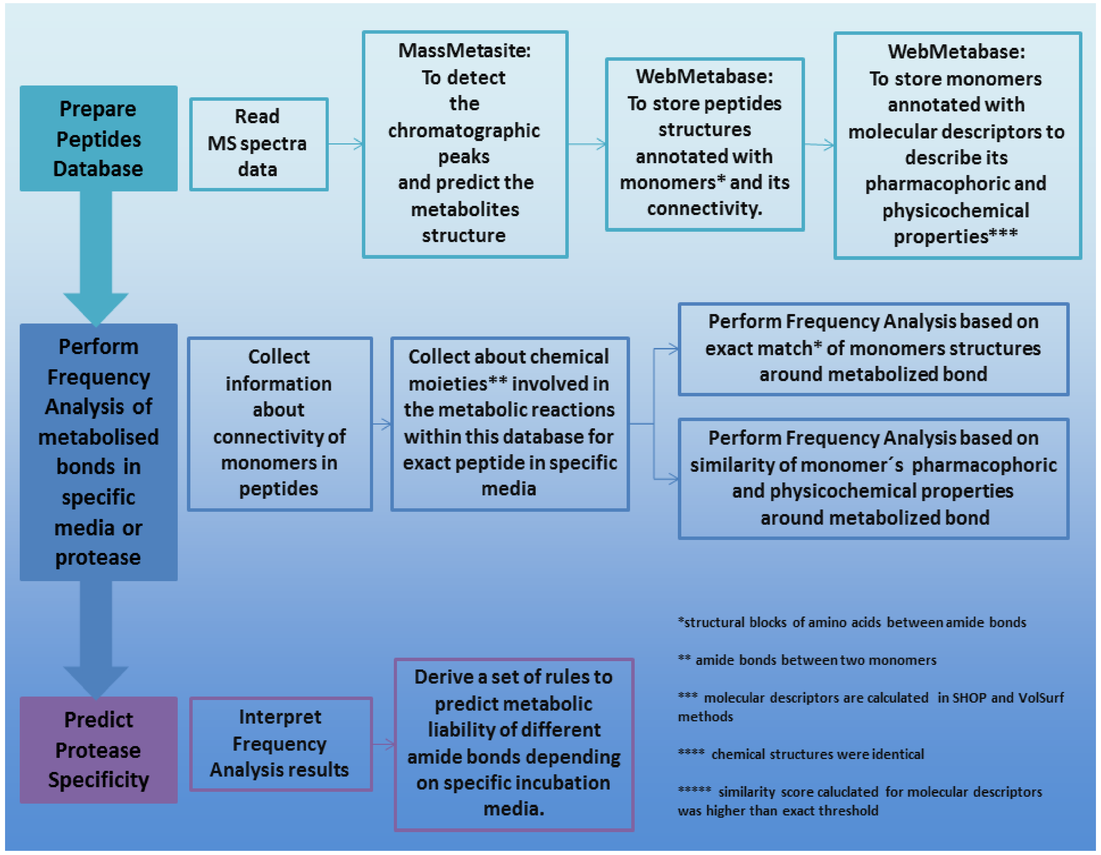
The main algorithm at the first stage uses MassMetaSite and WebMetabase to elucidate the metabolites structure. At the next step we stored peptides-parent and metabolites structure in the database annotated with monomers (blocks of amino acids between amide bonds) and its connectivity. In this way the development of a chemical aware searching algorithms for any kind of peptide and substructures of any size was enabled. Moreover each monomer was annotated with molecular descriptors to describe its pharmacophoric and physicochemical properties represented by that monomer, making it possible to perform a similarity search based on these properties. It is worth notice that the developed search algorithm was not based on the theoretical mass spectrum or sequence alignment, but rather in performing an exact search of peptides and its metabolites monomers connections.

For the following analysis as a pilot study we used the frequency analysis results of the using stored information we performed a frequency analysis of the chemical moieties (e.i. amide bonds between two monomers) involved in the metabolic reactions within this database. It was seen that there was 36 moieties, 13 bonds were met 2 times and 2 were met in the database 4 times. Amide bonds between Lysine and Leucine were met 4 time and were broken 4 times. Amide bond between Leucine and Serine was met 4 times and was broken 2 times.

Input Peptide Structure	Monomers	Bonds	Metabolised bonds	
			Broken Amide Bond	Amount of times met in DB/Broken
				2/3
				2/3
				1/1
				1/1
				1/1

Methods

We collected HRMS-driven experimental information about several experiments that consider different incubations conditions as well as several peptides (linear, cyclic and containing non-natural amino acids) incubated with the human recombinant expressed enzymes dipeptidyl-peptidase-IV (DPP-4) and neutral endopeptidase (NEP) as well as human hepatocytes and human lysosome model.



Conclusions

A new algorithm was developed to store information about peptide in a chemical aware searchable format and to perform search based on match of chemical structure or similarity of physicochemical and pharmacophoric proterties.

Moreover this approach is able to perform frequency analysis to the most metabolically liable amide bonds in peptides for the specific proteases and the specific medias.

Acknowledgements

MD MOLECULAR DISCOVERY

LMD
Lead Molecular Design s.l.