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HPLC Analysis and Drug Development Using Peptides

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ABSTRACT: In the last decade peptide drug development has made great progress. There have been many technological advancements in production, modification, and analytics. The drawbacks of peptides are overcome by using both chemical and biological methods and employing newer design and delivery strategies. Peptides bonds are multiple amino acids that are linked via amide bonds and peptide synthesis is the production of peptides compounds. Peptides are chemically synthesized by the condensation reaction of the carboxyl group of one amino acid to the amino group of another.

Huge variety of natural and modified peptides have been studied and obtained, covering multiple therapeutic areas. Therapeutic peptides research started with studies of natural human hormones, like vasopressin, oxytocin, insulin, and gonadotropin-releasing hormone (GnRH). The 20th century saw the discovery of several life-saving bioactive peptides, such as insulin and adrenocorticotrophic hormone. The discovery and development of insulin, a peptide with 51 amino acids is one of the milestones in the field. Since then the development of peptide drugs has been one of the hottest topics in pharmaceutical research with more than 80 peptide drugs all over the world.

High-performance liquid chromatography (HPLC) is used extensively for the isolation and purification of peptides. Protein molecules have large molecular weight and multiple functional groups, and hence their HPLC analysis is different from the analysis of small molecules. Therapeutic peptides have low production costs, sale prices, oral administration, and good membrane penetration ability.

KEYWORDS: High-performance liquid chromatography, Peptides, Protein, Amino acid, Hydrophobic

I. INTRODUCTION

High-performance liquid chromatography (HPLC) has seen many applications for the past two decades for the isolation and purification of peptides. HPLC modes for peptide separations, the value of various HPLC techniques for structural characterization of peptides like assessment of oligomerization state of peptides/proteins by size exclusion chromatography and monitoring the hydrophilicity/hydrophobicity of amphipathic -helical peptides are very important. High-performance liquid chromatography (HPLC) packings and instrumentation has developed so much that it has revolutionized the efficiency and speed of separation of molecules and peptides. Molecules possessing some degree of hydrophobic character, such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution.

I.I. PROTEINS

Protein molecules are large molecules with a molecular weight greater than 5000 Da. The primary amino acid sequence and tertiary structure of the target protein must be known in order to develop a HPLC method for proteins. The primary amino acid sequence gives the information about which functional groups are present in the molecule and in what quantity. Peptides and proteins interact with the chromatographic surface. Their retention time can be determined by the molecular composition of specific contact regions.

The figure shows four degrees of protein structure. When the protein molecule unfolds, interior hydrophobic amino acids side chains are exposed, which increases the retention time and decreases the separation efficiency. Stationary phase hydrophobicity, ion-pairing agents, detergents, mobile phase polarity, mobile phase pH, and oven temperature decide protein unfolding.

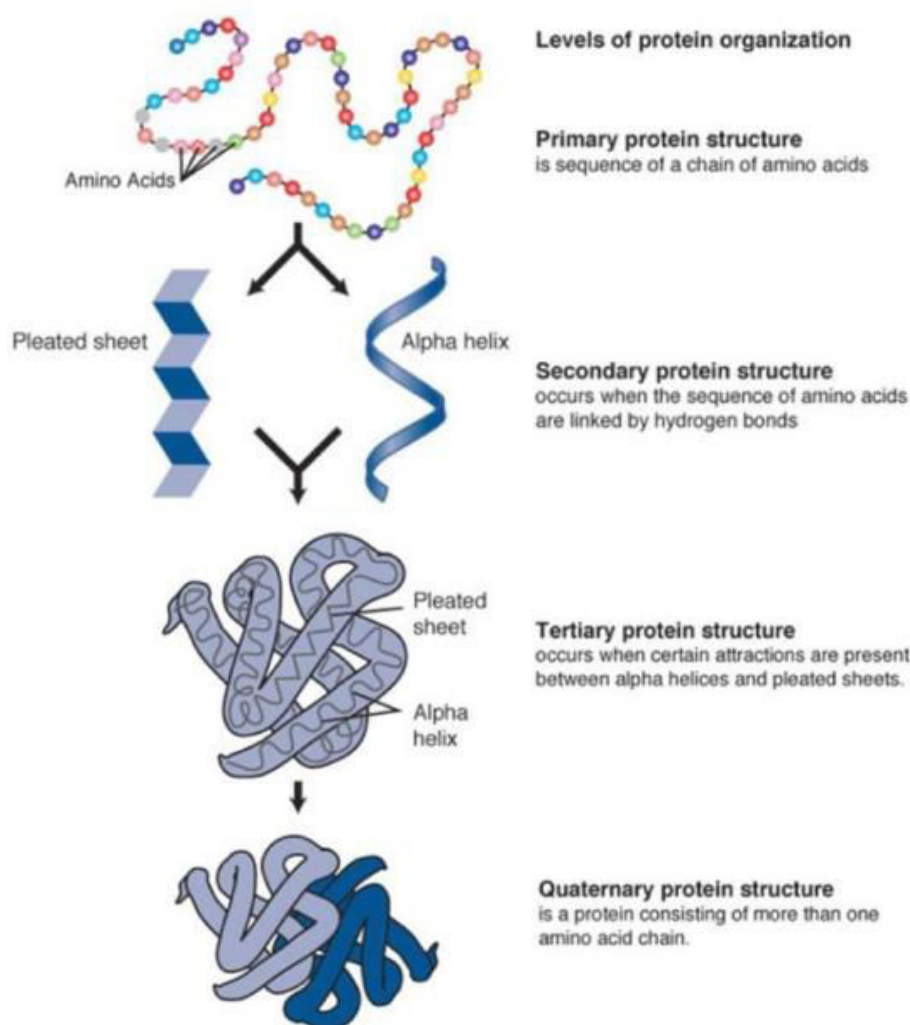


Figure 1: Four degrees of protein structure [1]

I.II PROPERTIES OF AMINO ACIDS

The side-chains of amino acids are classified according to their polarity, i.e., nonpolar or hydrophobic vs polar or hydrophilic. Further, the polar side-chains are divided into three main groups: uncharged polar, positively charged, or basic side-chains, and negatively charged or acidic side-chains. The size, shape, property of the side chain and peptide purification change within each group. Hydrophilicity/hydrophobicity and the number of charged groups present are important factors in the separation of peptides.

Intrinsic amino acid side-chain hydrophilicity/hydrophobicity coefficients and reversed-phase (RP)-HPLC derived values at pH 2.0 and pH 7.0 are shown in Table 1.

Table 1: [2]

Hydrophilicity/Hydrophobicity Coefficients Determined at 25°C by Reversed-Phase High-Performance Liquid Chromatography of Model Peptides at pH 2.0 and 7.0^a

Amino acid Substitution ^b	pH 2.0 ^c $\Delta t_R(\text{Gly})^d$	pH 7.0 ^c $\Delta t_R(\text{Gly})^d$
Trp	32.4	33.0
Phe	29.1	30.1
n-Leu	24.6	25.9
Leu	23.3	24.6
Ile	21.4	22.8
Met	15.7	17.3
n-Val	15.2	16.9
Tyr	14.7	16.0
Val	13.4	15.0
Pro	9.0	10.4
Cys	7.6	9.1
Ala	2.8	4.1
Glu^e	2.8	-0.4
Lys	2.8	-2.0
Thr	2.3	4.1
Asp	1.6	-0.8
Gln	0.6	1.6
Arg	0.6	4.1
Ser	0.0	1.2
His	0.0	4.7
Gly	0.0	0.0
Asn	-0.6	1.0
Orn	-0.6	-2.0

^a Taken from ref. 10.

^b The amino acid substitutions at position X in the peptide sequence Ac-X-G-A-K-G-A-G-V-G-L-amide; n-Leu, n-Val and Orn denote norleucine, norvaline and ornithine, respectively.

^c Conditions: see Subheading 3.3.1.

^d Δt_R is the difference in retention time relative to the Gly-substituted peptide.

^e Potentially charged residues (Asp, Glu, Arg, His, Lys, Orn) are in bold; the values between pH 2.0 and pH 7.0 that differ by more than 2 min are also in bold.

By “Intrinsic” it means that the maximum possible hydrophilicity/hydrophobicity of side-chains in the absence of nearest neighbor or conformational effects that would decrease the full expression of the side-chain hydrophilicity/hydrophobicity when the side-chain is in a polypeptide chain. This scale is the starting point to find the parameters.

Our work intends to analyze various possibilities of therapeutic drug discovery using peptides for Covid- 19 family of viruses and chart right ahead in drug discovery for further researchers who actually discover drugs.

II. RELATED WORK

In [5] Using a chromatographic technique (HPLC) and ideal experimental conditions, the scientists assessed the protein quality of a high protein raw component (corn gluten). They employed pre-column derivatization, separation by high-

performance liquid chromatography (HPLC), and UV detection in the investigation. The next step was to use the improved technique to identify the amino acids from raw, high-protein products that are frequently used in animal feeding (corn gluten, 61 percent CP). Six distinct days were dedicated to the analysis of a set of six samples, each of which was produced in two.

In [6] authors assess data from therapeutic drug monitoring (TDM) which gives the practitioner more understanding of the variables affecting the patient's response to pharmacological therapy. When a patient does not respond to a typical therapeutic dose, for instance, measuring the plasma level can help distinguish between a noncompliant patient and a real non-responder. TDM also offers helpful information on how drug use patterns vary from person to person and how drug use changes as a result of changes in physiological state or disease processes.

In [7] the RPLC, IEC, and SEC HPLC modes that can be used to analyze the required protein molecules are examined by the authors. When creating a method for protein molecule analysis, one should take into account the many factors included in these diverse modes. There are various detectors available for these various HPLC modes. Depending on the goal of the procedure and different protein properties, detectors can be chosen.

In [8] The authors review: (i) a comprehensive overview of peptide drug development and the underlying technologies used to date, and (ii) an up-to-date review of key developments in computational modeling peptide-protein interactions (PepPI) with the goal of helping experimental biologists exploit appropriate splicing methods to advance peptide interference strategies against PPIs. Despite recent advances in protein-protein and peptide-protein structural computational modeling, major challenges remain.

In [9] authors focused on the main viruses and servers goals which can guide efforts in pharmacogenomics discovery . New medicine for this terrible disease of SARS-CoV-2. In principle, all CoV enzymes and proteins involved in viral replication and control Cell hosts are potential drug targets in study treatment options for SARS-CoV-2. Given the urgency of the COVID-19 pandemic, authors feel the repurposing of approved drugs is the only alternative to find a timely effective treatment. Drugs currently under clinical trials were initially approved for other indications. However, the past and present coronavirus outbreaks require preparedness not only for the current situation but also for a future potential re-emergence of novel coronaviruses. In this sense, authors feel it is of utmost importance to design drugs acting as pan-coronavirus antivirals or through a multi-target approach to avoid a lack of effectiveness by viral mutation escape.

In [10] different modes of chromatography that can cover almost every separation case are considered. Additionally, an outlook to a very recent continuous chromatographic process (namely Multicolumn Countercurrent Solvent Gradient Purification, MCSGP) and future perspectives regarding purification strategies are considered.

In [11] authors summarize the efforts and achievements in peptide drug discovery, production, and modification and their current applications. They also discuss the value and challenges associated with future developments in therapeutic peptides. The discovery of potential therapeutic peptides is the first step in peptide drug development, followed by chemical or biological peptide synthesis and sequence modification to improve its pharmacological properties. Here authors summarize the fundamental technologies utilized for peptide production and modification.

Peptide drugs have been applied to a wide range of diseases, such as diabetes mellitus, cardiovascular diseases, gastrointestinal diseases, cancer, infectious diseases, and vaccine development. Considering their huge therapeutic potentials, market prospects, and economic values, we expect therapeutic peptides to continue to attract investment and research efforts and to achieve long-term success.

Our work intends to analyze various possibilities of therapeutic drug discovery using peptides therapeutic properties.

III. PROPOSED SOLUTION

The generally poor cell membrane permeability of peptides has limited their application against inaccessible intracellular targets. Peptide therapeutic development has largely focused on extracellular targets due to this limitation. Improvement of membrane permeability or development of strategies that facilitate active intracellular uptake will be critical for successful peptide-based targeting of intracellular PPIs. Potential strategies for improving intracellular

uptake include modulation of the hydrophobicity and electrostatic charges to improve passive uptake, or conjugation of the active drug

peptide to a cell-penetrating peptide (CPP) to facilitate its active transport.

Bioavailability of peptide biotherapeutics is usually greatly enhanced when they are more watersoluble, as effective serum concentrations can be easily maintained. Optimization of aqueous solubility is however a largely empirical process, with experimental identification of unnecessary hydrophobic amino acids which can be replaced with charged or polar residues to modulate the P_i while maintaining bioactivity.

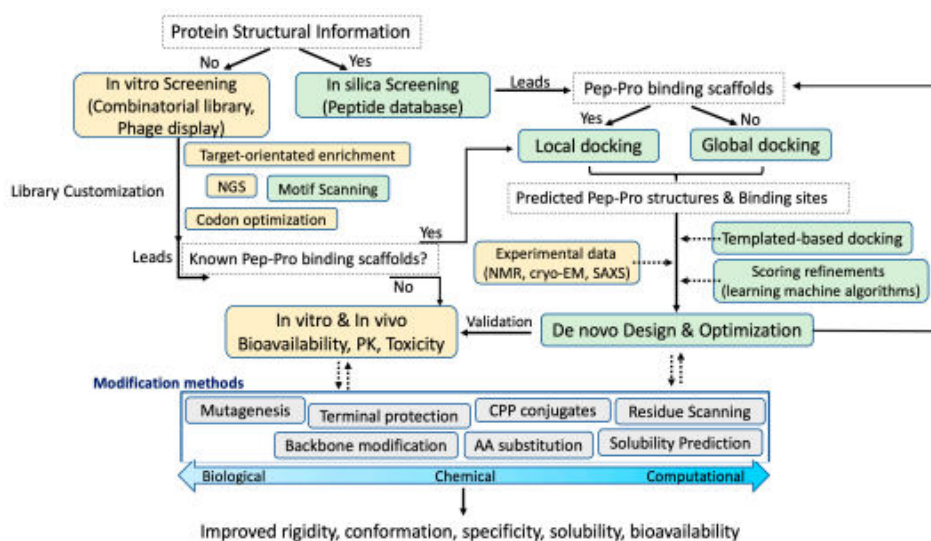


Figure 2 :Modular view of the peptide drug development cycle

This flowchart provides ways proposed for drug discovery. Boxes in green color indicate computational methods; gold are biological methods; grey are commonly modification methods applied for improving peptide bioactivity. The blue two-headed arrow represents the modification methods that are relatively more biological, chemical or computational. White dashed boxes are criteria for accessing which method can be chosen next depending on availability of information. Solid or dashed arrows indicate direct or optional connections between methods, respectively.

IV. CONCLUSION

The discovery of potential therapeutic peptides is the first step in peptide drug development, followed by chemical or biological peptide synthesis and sequence modification to improve its pharmacological properties.

Peptides have become a unique class of therapeutic agents in recent years as a result of their distinct biochemical characteristics and therapeutic potential. Although peptides outperform small molecules and large biologics in some aspects, they often suffer from membrane impermeability and poor stability in vivo, due to the intrinsic limitations of amino acids. This paper intends to analyze and provide proper pathway to therapeutic drug discovery using computational and other methods available and arrange it in a logical manner which would help further researchers.

REFERENCES

1. HPLC METHOD DEVELOPMENT FOR PROTEINS AND POLYPEPTIDES, Shyamala*, Vishnu Priya .P, Anjali Devi .N, ISSN 2319 – 5436, IJSIT, Volume 2, Issue 4, July-August 2013
2. HPLC Analysis and Purification of Peptides Colin T. Mant, Yuxin Chen, Zhe Yan, Traian V. Popa, James M. Kovacs, Janine B. Mills, Brian P. Triplet, and Robert S. Hodges, National Institutes of Health grant to R.S. Hodges (R01GM61855)



3. A Guide to the Analysis and Purification of Proteins and Peptides by Reversed-Phase HPLC, ACE columns
4. Therapeutic peptides: current applications and future directions, Lei Wang, Nanxi Wang, Wenping Zhang, Xurui Cheng, Zhibin Yan, Gang Shao, Xi Wang, Rui Wang and Caiyun Fu, www.nature.com/sigtrans, <https://doi.org/10.1038/s41392-022-00904-4>
5. Evaluation of the dietary protein using an optimized HPLC method, I. Maros, A. Untea, C. Dragomir, M. Olteanu, Biotechnology in Animal Husbandry 27 (3), p 1345-1355, 2011 ISSN 1450-9156 Publisher: Institute for Animal Husbandry, Belgrade-Zemun
6. Therapeutic drug monitoring and HPLC: A review, Prashanth P, Navin Patil, Suneel Kumar Reddy, Somashekar HS, Narendranath S, Geetha MPharmacologyonline 3: 1176-1191 (2011)Newsletter
7. HPLC method development for proteins and polypeptides, Shyamala*, Vishnu Priya .P, Anjali Devi .N, IJSIT (www.ijsit.com), Volume 2, Issue 4, July-August 2013.
8. A Comprehensive Review on Current Advances in Peptide Drug Development and Design, Andy Chi-Lung Lee, Janelle Louise Harris, KumKum Khanna and Ji-Hong Hong, Int. J. Mol. Sci. 2019, 20, 2383; doi:10.3390/ijms20102383
9. COVID-19: Drug Targets and Potential Treatments, Carmen Gil,*Tiziana Ginex, Ines Maestro, Vanesa Nozal, Lucía Barrado-Gil, Miguel Ángel Cuesta-Geijo, Jesus Urquiza, David Ramírez, Covadonga Alonso, Nuria E. Campillo, and Ana Martinez*, <https://dx.doi.org/10.1021/acs.jmedchem.0c00606> J. Med. Chem. 2020, 63, 12359–12386
10. De Luca, C.; Lievore, G.; Bozza, D.; Buratti, A.; Cavazzini, A.; Ricci, A.; Macis, M.; Cabri, W.; Felletti, S.; Catani, M. Downstream Processing of Therapeutic Peptides by Means of Preparative Liquid Chromatography. Molecules 2021, 26, 4688. <https://doi.org/10.3390/molecules26154688>
11. Therapeutic peptides: current applications and future directions, Lei Wang, Nanxi Wang, Wenping Zhang, Xurui Cheng, Zhibin Yan, Gang Shao, Xi Wang, Rui Wang and Caiyun Fu, Signal Transduction and Targeted Therapy (2022) <https://doi.org/10.1038/s41392-022-00904-4>
12. Abraham T. Girgih, Chibuike C. Udenigwe, Fida M. Hasan, Tom A. Gill, Rotimi E. Aluko., Antioxidant properties of Salmon (Salmosalar) protein hydrolysate and peptide fractions isolated by reverse phase HPLC, Food Research International, 2013; 52 (1) : 315-22.
13. Colin T. Mant, T.W. Lorne Burke, Nian E. Zhou, J.M. Robert Parker, Robert S. Hodges., Reversed-phase chromatographic method development for peptide separations using the computer simulation program predigest.t, Journal of Chromatography, 1989; 485: 365-82
14. Andrea Penwell, Kendall Sharp, Marc Mansour, Leeladhar m Sammat. Development and validation of an HPLC/UV assay for separation and quantification of peptide antigens from a liposomal vaccine delivery platform, Journal of Pharmaceutical and Biomedical Analysis, 2012; 66 : 176-82
15. O.V. Krokhin, R. Craig, V. Spicer, W. Ens. et.al., Retention Times of Tryptic peptides in Ion Pair Reversed-phase HPLC, Molecular & Cellular Proteomics, 2004; 3: 908-19.



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