AAPS Short Course: Characterization Methods for Aggregates and Particles in Peptide and Small Protein Formulations

Sunday, October 25, 2015
Orange County Convention Center
Orlando, Florida
Logistics
AAPS Short Course: Characterization Methods for Aggregates and Particles in Peptide and Small Protein Formulations

• To explore and identify analytical methods to characterize aggregation and fibrillation in peptide formulations.

• To provide a collaborative, cross-disciplinary forum for scientific discussion.

• To provide a regulatory perspective on biophysical phenomena in peptide and small protein formulations.
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Overview on Peptide Stability

Nathalie Y. Toussaint, Ph.D.
Principal Scientist
Discovery Pharmaceutical Science
Merck Research Labs
Renaissance of Peptide Drugs: Science and Invention

- Renaissance means rebirth; having an acquired profound knowledge or proficiency in multiple fields.
- Since 2001, over 19 new therapeutic peptides have been approved in the U.S:
  - 8 approved in 2012
  - Many pharmaceutical companies have clinical trials ongoing.
- Novel peptide therapeutics have the potential to fulfill unmet medical needs and enhance patients' life.

Peptide Therapeutics Revenue 2010-2018

Actual and Projected Peptides Therapeutics Revenue

Therapeutic Areas for Peptides in Clinical Trials*

*Data from February 2013
Peptide and Small Protein Structural Diversity

Relative Molecular Mass (Daltons)

- Celecoxib
- Glucagon
- Insulin
- Monoclonal antibody

Peptides and small proteins
Amino Acids: Basic Structure

General Structure

Amino Acid Structure

Isomerism

Zwitterion

Side chains

R-group
(variant)

https://biochemanics.wordpress.com/category/amino-acids/
Protein structure governs function/activity

Secondary Structure: helix and pleated sheets

Tertiary Structure: folded helix and pleated sheets

Quaternary Structure: two or more polypeptides in their folded states

Hydrogen bonds

Pleated Sheet

Hydrophobic interaction

Polypeptide strand

Disulfide bridge between Cys

Ionic bond

Details of bonds associated with tertiary structure

2010 Pearson Education, Inc
20 Natural Amino Acids

Small

- Glycine (Gly, G)
  MW: 57.05

- Alanine (Ala, A)
  MW: 71.09

- Serine (Ser, S)
  MW: 87.08, pKₐ ≈ 16

- Threonine (Thr, T)
  MW: 101.11, pKₐ ≈ 16

- Cysteine (Cys, C)
  MW: 103.15, pKₐ = 8.35

Hydrophobic

- Valine (Val, V)
  MW: 99.14

- Leucine (Leu, L)
  MW: 113.18

- Isoleucine (Ile, I)
  MW: 113.16

- Methionine (Met, M)
  MW: 131.19

- Proline (Pro, P)
  MW: 97.12

Aromatic

- Phenylalanine (Phe, F)
  MW: 147.18

- Tyrosine (Tyr, Y)
  MW: 163.18

- Tryptophan (Trp, W)
  MW: 186.21

- Aspartic Acid (Asp, D)
  MW: 115.09, pKₐ = 3.0

- Glutamic Acid (Glu, E)
  MW: 129.12, pKₐ = 4.67

Acidic

- Asparagine (Asn, N)
  MW: 114.11

- Glutamine (Gln, Q)
  MW: 128.14

- Histidine (His, H)
  MW: 137.14, pKₐ = 6.04

- Lysine (Lys, K)
  MW: 128.17, pKₐ = 10.79

- Arginine (Arg, R)
  MW: 156.19, pKₐ = 12.48

Basic

- Asparagine (Asn, N)
  MW: 114.11

- Glutamine (Gln, Q)
  MW: 128.14

- Histidine (His, H)
  MW: 137.14, pKₐ = 6.04

- Lysine (Lys, K)
  MW: 128.17, pKₐ = 10.79

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Amide
Routes of Peptide & Protein Instability

- Isomerization
- Aggregation
- Deamidiation
- Fibrillation
- Oxidation
- Denaturation
- Hydrolysis
- Adsorption
- Disulfide Exchange
- Microbial contamination
Key Regulatory CMC Considerations for Peptide and Small Proteins

- Drug substance manufacturing and controls
- Drug substance characterization
- Drug product manufacturing and controls
- Drug product stability
- Safety (immunogenicity)

No official guidelines for peptide drugs
Key Take Aways

• All amino acids have this basic structure, differing only in the structure of the R-group
  – The R-Group give rise to the AA properties
  – Protein Structure Governs Function/Activity

• Peptide stability can be broadly classified as:
  – Chemical stability, referring to modifications of peptide or protein via chemical bonds
  – Physical instability can lead to immunogenicity, altered bioperformance and product quality concerns.

• Several analytical techniques are often utilized to characterize peptides/proteins completely to examine its stability profile
Acknowledgements

Deep gratitude to the many Merck colleagues who contributed to strategic guidance including:

- Annette Bak
- Pete Wuelfing
- Ellen C. Minnihan
- Grace Okoh
- Heidi Ferguson
- Jenna Terebetski
- Dennis Leung
- Tomi Sawyer
Questions?
Peptide Stability Assessment

- Thermal Analysis
- Spectroscopy
- Bioassay
- Immunoassay
- Electrophoresis
- Chromatography
Analytical Methods: Thermal Analysis

- Differential Scanning Calorimetry (DSC)
  - Microcalorimetry
  - Modulated DSC (MDSC)
    - Converts total heat flow into heat capacity component and kinetic component

http://www.malvern.com
Analytical Methods: Spectroscopy

- Ultraviolet Spectroscopy
- Fluorescence Spectroscopy
- Nuclear Magnetic Resonance (NMR)
- Circular Dichroism Spectroscopy (CD)
- Dynamic Light Scattering
- Colorimetric Assay

Mira et al. BMC Structural Biology 2004 4:7
Wyatt.com; Joachim Pietzsch nature.com
Analytical Methods: Immunoassays and Bioassays

**Bioassays**
- Measurement of the pharmacological activity/function
- Determination of the side-effect profile, including the degree of drug toxicity
- Determining the specificity of certain enzymes to certain substrates

**Immunoassays**
- Biochemical test that measures the macromolecule in a solution through the use of an antibody
- Enzyme-linked immunosorbent assays (ELISAs), employ the use of detection enzymes
Analytical Methods: Electrophoresis

- **SDS PAGE** (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis)
  - Denatures protein
- **IEF** (isoelectric focusing)
  - Commonly used for pI
- **Capillary Electrophoresis**
  - Allows for the dissipation of heat

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http://www.labplan.ie/3page.asp?menu=191&page=703&subpage=91
Biochemistry, 7th Edition 2012 W.H. Freeman and Company
Analytical Methods: Chromatography

- Hydrophobic Interaction Chromatography (HIC)
- Size exclusion chromatography (SEC)
- Ion exchange chromatography
- Affinity chromatography

(Nelson & Cox, Lehninger Principles of Biochemistry, 3rd ed)
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