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REPORT

ON

STABILITY OF POLYPEPTIDES AND PROTEINS

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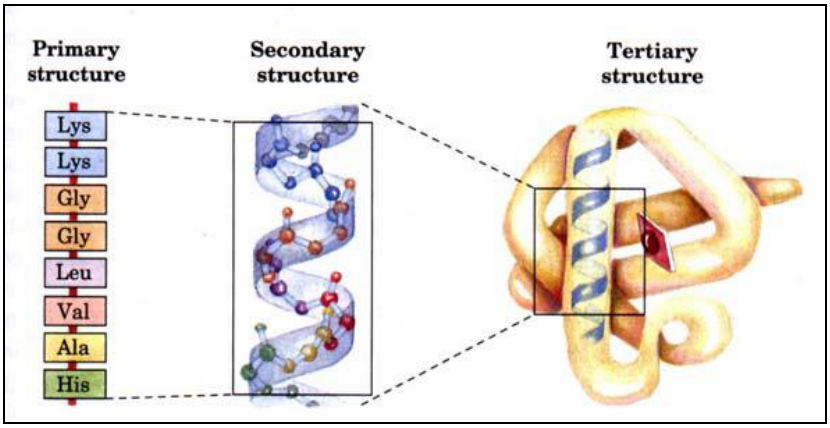
Stability of Polypeptides and Proteins

Background:

Proteins comprise an extremely heterogeneous class of biological macromolecules. They are often unstable when not in their native environments, which can vary considerably among cell compartments and extracellular fluids. Their properties make them particularly difficult to formulate but, with right approach, they can be developed into effective therapies. Proteins and polypeptides are fast becoming an important segment of the pharmaceutical industry. Although there have been tremendous advances in production of the active pharmaceutical ingredient (API), production of the peptide-based drug products is still a significant challenge.

Peptides are defined as polypeptides of less than 50 residues or so and lacking any organized tertiary or globular structure. Some do adopt secondary structure, although this tends to be limited, for example a single turn of an α-helix. While their smaller size makes them easier to deliver across biological barriers than larger proteins, their formulation can be problematic.



Mainly because of their chemical instability or degradation like by hydrolysis and racemization and physical degradation depending upon their molecular weight, they undergo denaturation, aggregation and precipitation; they are very challenging to be formulated in desired dosage form.

Proteins and peptides exhibit the following challenges to the formulation scientists:

- They exhibit maximal chemical instability.

- They tend to self associate.
- They adopt multiple conformers.
- They can exhibit complex physical instabilities, such as gel formation.

Chemical and physical properties of peptides and proteins have been studied extensively and the thermodynamics of protein structure have also been studied in detail and reported. But because of the complicated degradation mechanisms, it is generally more difficult to predict the stability of peptide and protein pharmaceuticals.

Proteins and peptides undergo degradation by two mechanisms:

a) Physical mechanisms
b) Chemical mechanisms

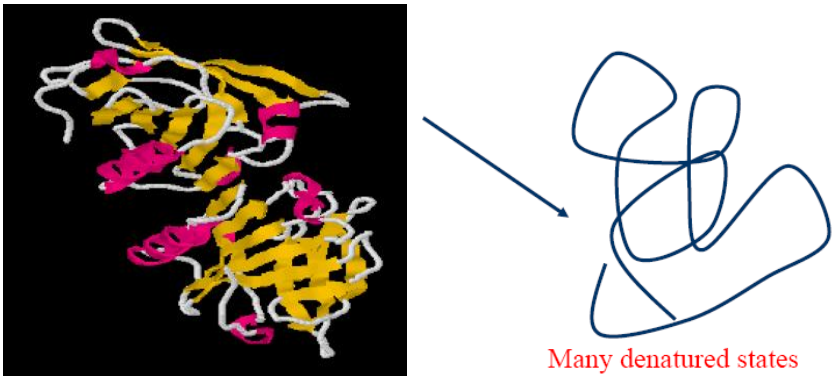
PHYSICAL INSTABILITY:

Physical instability or noncovalent changes are generally observed in case of larger peptides and proteins. Physical degradation includes ***denaturation, self association, aggregation, adsorption, and gelation.***

Denaturation: protein Denaturation is mainly associated with any modification in conformation not accompanied by rupture of peptide bonds and ultimate step might correspond to a totally unfolded polypeptide structure which can be reversible or irreversible. It can also results in loss of bioactivity mainly because of the alteration the tertiary structure of the proteins. Furthermore, exposure of hydrophobic groups upon Denaturation often leads to adsorption on the surfaces, aggregation, and precipitation. Denaturation sometimes also triggers the chemical degradation pathways often not seen with the native or natural tertiary (and/or quaternary) structure. Other effects of Denaturation are:

- Decreased solubility
- Altered water binding capacity
- Destruction of toxins

- Improved digestibility
- Increased intrinsic viscosity
- Inability to crystallize



Denatured proteins

Causes of protein Denaturation:

1. Temperature fluctuation
   - Effect of increased temperature:
     - Affect interactions of tertiary structure
     - Increased flexibility → reversible
     - H-bonds begin to break → water interaction
     - Increased water binding
     - Increased viscosity of solution
     - Structures different from native protein

   - Effect of decreased temperature:
     - Can result in Denaturation(for e.g.Gliadins, egg and milk proteins)
     - Remain active(for e.g.Some lipases and oxidases and Release from sub-cellular compartments)
     - Proteins with high hydrophobic/polar amino
     - residues and structures dependent on hydrophobic interactions

2. Water content affects heat Denaturation
3. Mechanical treatments
4. Hydrostatic Pressure
5. Irradiation
6. Heavy metal salts act to denature proteins in much the same manner as acids and bases. **Heavy metal salts** usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.
7. Heavy metals may also disrupt disulfide bonds because of their high affinity and attraction for sulfur and will also lead to the denaturation of proteins

Self association: The propensity of peptides to ***self-associate*** is connected with their physical instability. While self-association of peptides for e.g. melittin and corticotrophin – releasing factor (CRF), the relationship between these metastable oligomeric species and larger aggregates has been investigated, but still unclear. Noncovalent aggregation has been suggested for many other proteins, but not always confirmed. For e.g. a conjugate formed between a vinca alkaloid and a monoclonal antibody exhibited aggregation in solution, the mechanism of which (covalent or noncovalent) was not clear. Aggregates formed upon agitation of insulin solutions in the presence of hydrophobic surfaces (Teflon) were dissociated with urea, suggesting noncovalent aggregation.

Aggregation can lead to either amorphous or ordered forms. Ordered aggregates usually take the form of fibrils; these fibrillar structures are the basis for the most common type of the aggregation seen for peptides, namely gelation.Gelation is the last step in a pathway that starts with the formation of peptides protofibrils that exhibit β-sheet structure. The protofibrils then associate to form mature fibrils, which propagate and intertwine, resulting in gelation.

Detection of aggregates: Insoluble aggregates can be detected by FTIR, Raman, and electron spin resonance spectroscopy, or light scattering techniques (UV absorption). Soluble aggregates can be detected by HP-SEC (High Performance Size Exclusion Chromatography), found in many proteins like hGH, insulin, interferon-2 (IL-2), anti trypsin-a1,IFN-g, basic fibroblast growth factor and IFN-b.

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