Pharmaceutical Biotechnology Ideathon

Drug development and Delivery systems

Problem statement: Aggregate formation in high protein concentration formulations is known. How can it be prevented?

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Overall Objective

- Understanding the root cause for aggregation that takes place in high protein concentration formulations.
- Research on how to prevent aggregation / controlled aggregation mechanisms.

State of Art

Protein aggregation occurs in vivo as a result of improper folding or misfolding. Diverse diseases arise from protein misfolding, including most of the neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, the prion encephalopathies and Huntington's disease,etc.

The aggregates are usually arranged into structurally well-defined fibrils that create amyloid deposits. The structural instability of amyloidogenic proteins is caused by mutations, post-translational changes, or local circumstances like pH, temperature, and co-solutes, among other things.

- Aggregates are a risk factor for patients' immune responses. Aggregation is difficult to reverse by dilution or changing pH.
- Environmental conditions during production, processing, transportation and storage of therapeutic protein can enhance aggregation by affecting either (or both) conformational and colloidal stability.
- Presence of any aggregation in a protein pharmaceutical can impact activity, solubility, and may even present protein specific immune responses, and is generally not acceptable for product release.

State of Art (contd.)

There is no single protein aggregation pathway but a variety of pathways, which may differ between proteins and may result in different end states.

Protein aggregates have been categorized into the following categories:

- by type of bond: noncovalent aggregates (bound by weak electrostatic forces) versus covalent aggregates (e.g., caused by disulfide bridges)
- by reversibility: reversible versus irreversible aggregates
- by size: small soluble aggregates (oligomers) such as dimers, trimers, tetramers, etc. versus large ≥10-mer oligomers versus aggregates in the diameter range of some approx. 20 nm to approx. 1 µm versus insoluble particles in the 1–25 µm range versus larger insoluble particles visible to the eye under defined inspection conditions
- by protein conformation: aggregates with predominantly native structure versus aggregates with predominantly nonnative structure (i.e., partially unfolded multimeric species, fibrillar aggregates).

Lacuna in the field

Protein drugs are not currently available for oral intake (capsules and tablets) due to their larger size.

The only available solution is liquid forms (injections)

These routes allow very less volume per dose(~1 ml) and comparatively larger doses(~200 mg) are required for protein drugs thus high concentration(~10^2 mg/ml) is inevitable in many cases leading to aggregation.

Protein stability can be increased by the use of sugars and amino acids but the mechanism by which they promote folding can reduce the solubility of proteins.

Specific Aims

Determine the cause of aggregation and quantify the types of aggregates and achieve an optimal formulation

To suggest mechanisms to prevent aggregation and find a way to stabilise the API using the appropriate additive or excipient.

Maintaining the conformational and colloidal stability of biotherapeutic.

Optimization of the final excipient matrix most appropriate for each individual protein.

Methods

Quick summary on ways to control protein aggregation

- pH
- Temperature
- Salt concentration
- Additives- Amino acid, reducing agent, adding ligands, non-denaturing detergents etc.
- Maintain low protein concentration

- Proteins can be stabilized by the addition of excipients: Excipients can be broadly divided into the following types: buffers, sugars, polyols, polymers, surfactants, amino acids, amines, cyclodextrins, metal ions and salts.
- Polymers have recently been recognized as efficient alternatives to small molecules for the inhibition of protein aggregation, as these have great potential to combat aggregation-induced issues and are effective in inhibiting protein denaturation and facilitating refolding.
- Polyethylene glycol (PEG) derivatives. PEG in its native form is used for a number of applications such as drug delivery, as a laxative, and in wound healing. In protein research, PEG has been used to conjugate proteins, to protect them from degradation, increase their circulation time, and minimize the immune response.

- Pullulan-based nanogels. Akiyoshi et al. have published a series of reports using pullulan-based nanogels as artificial chaperones for the protection of proteins. They reported the synthesis of hydrophobic pullulan, which could self-assemble in water to form nanogels.For this, 1,6-diisocyanatohexane modified cholesterol was synthesized and made to react with pullulan to obtain cholesterol modified pullulan (CHP), which is capable of self-assembling in water to form a nanogel.
- Addition of surfactants to prevent adsorption of hydrophobic regions of proteins on the interfacial surfaces so that exposure of hydrophilic regions to the aqueous solution does not cause enhance aggregation. Eg - Poloxamer 88, polysorbate 20, 2-hydroxypropyl-beta-cyclodextrin.
- Preferential exclusion of protein by sugar and polyhydric alcohols to improve conformational stability by preferentially hydrating the protein thereby preventing exposure of hydrophobic regions that primarily cause aggregation. Possible drawback is Maillard reaction induced between sugars and amine groups.
- Solvents - Kosmotropic solvents promote the formation of hydrogen bonds within water, preventing hydrophilic regions of protein to bond with water - promotes formation of aggregation (eg – NaCl). Chaotropic solvents do the opposite and destabilise protein aggregates (eg – urea).
- Arginine-mediated stabilization - interacts with hydrophobic residues such as Tryptophan and reduces the overall hydrophobicity of the protein.
- Environmental conditions - A high pressure in combination of a high temperature (at 2 kbar at 70 °C) was shown to be effective in disaggregating growth hormone aggregates and even particulates. However, the high temperature condition may be used with caution, as it may promote chemical degradations.
- During formulation industrial process, regular in-process checks must be conducted. Avoiding continuous contact stirring during handling, transport as well as administration.

IDEA

- These concepts and technologies include use of uncommon/combination of formulation stabilizers, conjugation or fusion with potential stabilizers, site-specific mutagenesis, and preparation of nontraditional types of dosage forms—semiaqueous solutions, nonfreeze-dried solid formulations, suspensions, and other emerging concepts.

  Nano-systems for delivery of therapeutic proteins can help reduce aggregation. This area is relatively less researched.

- Encapsulation in dendrimers can also prevent proteins from aggregating as their self-interactions within the branched structure will be limited. Optimizing the concentration of dendrimer according to the type of therapeutic protein will be necessary to design the drug delivery system.

  Interaction between PAMAM dendrimer and bovine insulin hormone showed that the dendrimer did not aggregation among the hormone proteins.

- Additionally, dendrimer-based systems are being researched to increase oral bioavailability of drugs, thereby producing the scope for oral administration of protein therapeutics too.

Expected Outcomes

- A final formulation where the protein therapeutic is encapsulated in the dendrimer and once the drug has been released into the bloodstream, there are no signs of aggregation.
- No undesirable adverse side effects due to the conjugate system
- Stability of protein is retained in terms of conformation as well as functionality

Future Directions

- Experimental approaches that can be taken into consideration – total surface area and/or particle number, or approaches that saturate the immunological responses generated by each size of aggregate to check if one has a higher maximum induction than the other. It would be able to explore whether particular size ranges are inherently more immunogenic than others, as well as the immunological systems involved, using these experimental designs.
- PEGylation has also been found to lower immunogenicity of proteins due to the reported protection against aggregation. A possible alternative and more biocompatible option to PEGylation is glycosylation. Both colloidal and conformational stability has been shown to be enhanced by glycosylation, which would perhaps result in reduced aggregation.

- A practical approach to investigating more representative aggregates that occur in a clinical setting would be to investigate biotherapeutics samples that have passed their shelf life, for the occurrence of immunogenic aggregates.

- Use conformation-specific antibodies to recognize specific structures in aggregation intermediates.

- Develop methods to measure protein homeostasis and quality control.

Backup Plan

Protein drugs can be formulated at lower concentrations or larger volumes that can be cost-effective

Alternative drugs such as synthetic analogues of proteins may be engineered but bearing in mind the effectiveness and immuno-genecity

Identify different methods to cut down on the aggregation.

- Computational methods can predict aggregation-prone regions using sequence or structure input. The ability to identify aggregation hotspots has been facilitated by the development of at least 40 different algorithms.
- Rational design involves introducing specific mutations into a protein and subsequent analysis of the mutational effect in comparison to the behaviour of the wild-type protein.
- Directed evolution and in vivo screening methods obviate protein purification and large numbers of variants can be screened to identify proteins with enhanced properties.
- Deep mutational scanning can potentially sample every possible mutation and enables quantification of the effect on protein stability or aggregation to be determined in vivo

References

- Mahler, H.-C., Friess, W., Grauschopf, U., & Kiese, S. (2009). Protein aggregation: Pathways, induction factors and analysis. Journal of Pharmaceutical Sciences, 98(9), 2909–2934. doi:10.1002/jps.21566
- https://info.gbiosciences.com/blog/tips-for-preventing-protein-aggregation-loss-of-protein-solubility
- Jain, K., Salamat-Miller, N. & Taylor, K. Freeze–thaw characterization process to minimize aggregation and enable drug product manufacturing of protein based therapeutics. Sci Rep 11, 11332 (2021). https://doi.org/10.1038/s41598-021-90772-9
- Maggio, Edward. (2010). Use of excipients to control aggregation in peptide and protein formulations. Journal of Excipients and Food Chemicals. 1.
- https://pubs.rsc.org/en/content/articlehtml/2021/ma/d0ma00760a
- Roberts CJ. Protein aggregation and its impact on product quality. Curr Opin Biotechnol. 2014;30:211–217. doi:10.1016/j.copbio.2014.08.001
- Rajan, R., Ahmed, S., Sharma, N., Kumar, N., Debas, A., & Matsumura, K. (2021). Review of the current state of protein aggregation inhibition from a materials chemistry perspective: special focus on polymeric materials. Materials Advances, 2(4), 1139–1176. doi:10.1039/d0ma00760a

- Calamai, M., Canale, C., Relini, A., Stefani, M., Chiti, F., & Dobson, C. M. (2005). Reversal of Protein Aggregation Provides Evidence for Multiple Aggregated States. Journal of Molecular Biology, 346(2), 603–616. doi:10.1016/j.jmb.2004.11.067
- Wang, W., & Roberts, C. J. (2018). Protein aggregation – Mechanisms, detection, and control. International Journal of Pharmaceutics, 550(1), 251–268. https://doi.org/https://doi.org/10.1016/j.ijpharm.2018.08.043
- Moelbert, S., Normand, B., & De Los Rios, P. (2004). Kosmotropes and chaotropes: Modelling preferential exclusion, binding and aggregate stability. Biophysical Chemistry, 112(1), 45–57. https://doi.org/10.1016/j.bpc.2004.06.012
- Wang, Wei. "Advanced protein formulations." Protein science : a publication of the Protein Society vol. 24,7 (2015): 1031-9. doi:10.1002/pro.2684
- Lundahl, Mimmi L E et al. "Aggregation of protein therapeutics enhances their immunogenicity: causes and mitigation strategies." RSC chemical biology vol. 2,4 1004-1020. 4 May. 2021, doi:10.1039/d1cb00067e
- Nowacka, O., Milowska, K., & Bryszewska, M. (2015). Interaction of PAMAM dendrimers with bovine insulin depends on nanoparticle end-groups. Journal of Luminescence, 162, 87–91. https://doi.org/https://doi.org/10.1016/j.jlumin.2015.02.014
- Roberts, Christopher J. "Protein aggregation and its impact on product quality." Current opinion in biotechnology vol. 30 (2014): 211-7. doi:10.1016/j.copbio.2014.08.001

Thank you.