Conference abstract PDD05

Human Growth Hormone – Insights into its Aggregation Behavior using Denaturation Pathway Characterization

J. Wiesbauer¹,², S. Leitgeb¹,², B. Nidetzky²

¹ Research Center Pharmaceutical Engineering GmbH, Graz, Austria
² Institute of Biotechnology and Biochemical Engineering, University of Technology Graz, Graz, Austria
E-mail: johanna.wiesbauer@rcpe.at (J. Wiesbauer)
Pharm. 2010; 78: 594 doi:10.3797/scipharm.cespt.8.PDD05

Protein denaturation processes involving aggregation are among the prime factors impeding the development of stable protein drug formulations. Not only does aggregation limit the shelf-life of protein pharmaceuticals and potentially decreases the overall efficacy of therapeutic, it may also cause unwanted side effects such as immune reactions. Denaturation kinetically coupled to aggregation can occur in all stages of the production process and its prevention constitutes a major effort in biopharmaceutical technology. A problem in designing rational strategies counteracting the aggregation is that a sound molecular basis underlying the denaturation process is usually not available. Dissecting the overall denaturation pathway into discrete kinetic steps would allow one to evaluate stabilizing effects of certain process conditions more systematically. Generally, understanding of aggregation during or induced by the manufacturing process is an important goal to be achieved by the pharmaceutical industry.

For our studies we used human growth hormone (hGH), which is a common therapeutic protein, and examined its aggregation mechanism under a range of process conditions. hGH is a single domain, globular protein consisting of 191 amino acids and has a molecular mass 22 kDa. Its tertiary structure shows 4 antiparallel α-helices and two disulfide bridges (53 to 165 and 182 to 189).

Experiments were carried out under accelerated aggregation conditions. We show that the gas/liquid interface generated by aeration is strongly promoting aggregation. Stirring likewise resulted in aggregation. Results from SEC measurements and native or SDS PAGE of time-course experiments should help understanding aggregation and its kinetic pathway. This may help in developing more stable drug formulations.

This work was supported by FFG, Land Steiermark and Steirische Wirtschaftsförderung (SFG)