Human growth hormone – insights into its aggregation behavior using denaturation pathway characterization

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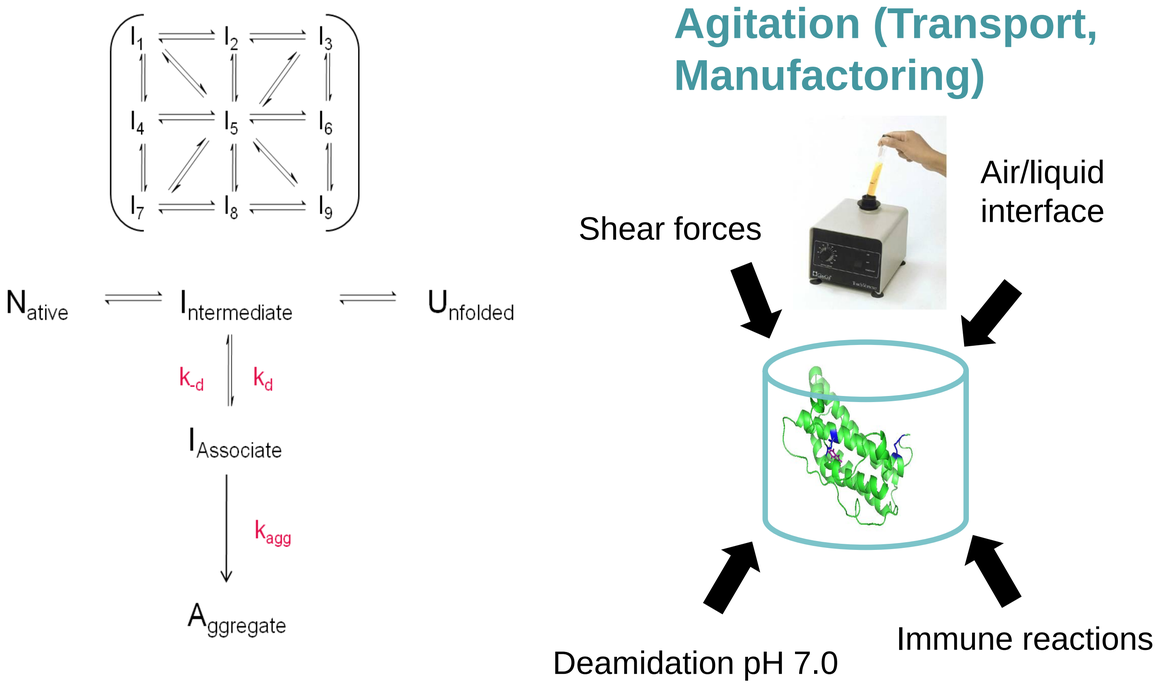
Introduction

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.

hGH and aggregation



[1] DeFelippis et al. *Biochemistry* (1993) 32, 1555-1562

External effects and aeration

The use of N_2 instead of compressed air did not influence the reaction. The use of Poloxamer 188 [2] resulted in a stabilized solution, whereas the reduction of protein concentration by 10-fold destabilized the system [3].

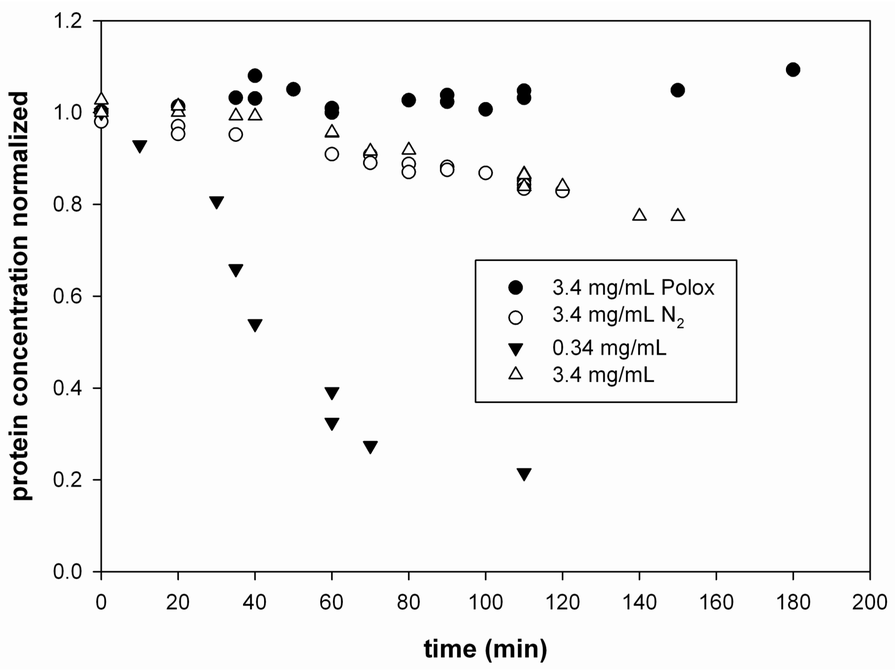


Figure 2: Influence of external factors like excipients, protein concentration or gas used on the loss of protein during aertion.

[2] Katakam and Banga. *Pharm Dev Technol* (1997) 2, 143-149
[3] Treuheit et al. *Pharm Res* (2002) 19, 511-516

Conclusion

- ✓ hGH is sensitive to air liquid interfaces.
- ✓ The loss of protein due to aggregation is dependent on the aeration rate.
- ✓ External effects and their influence on the stability of hGH during aeration

Poloxamer 188	Low protein concentration	Aeration with N_2	Foam formation
+	-	No effect	No effect

Air/liquid interface

In previous experiments it was found that hGH is highly sensitive to air-liquid interfaces. So aeration studies at different flow rates were started with compressed air and 3.4 mg/mL hGH.

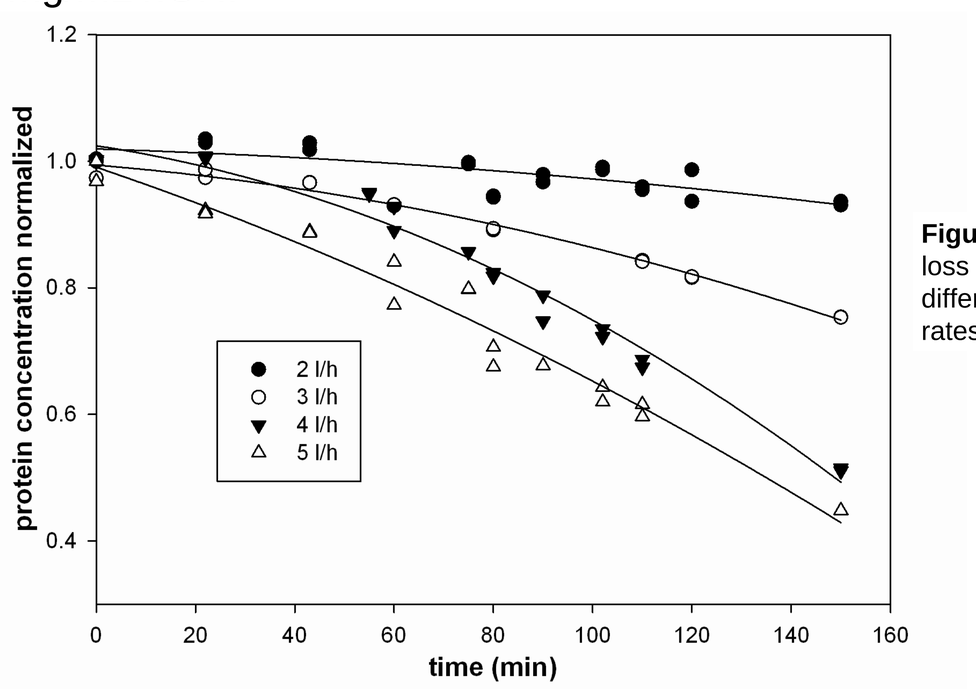


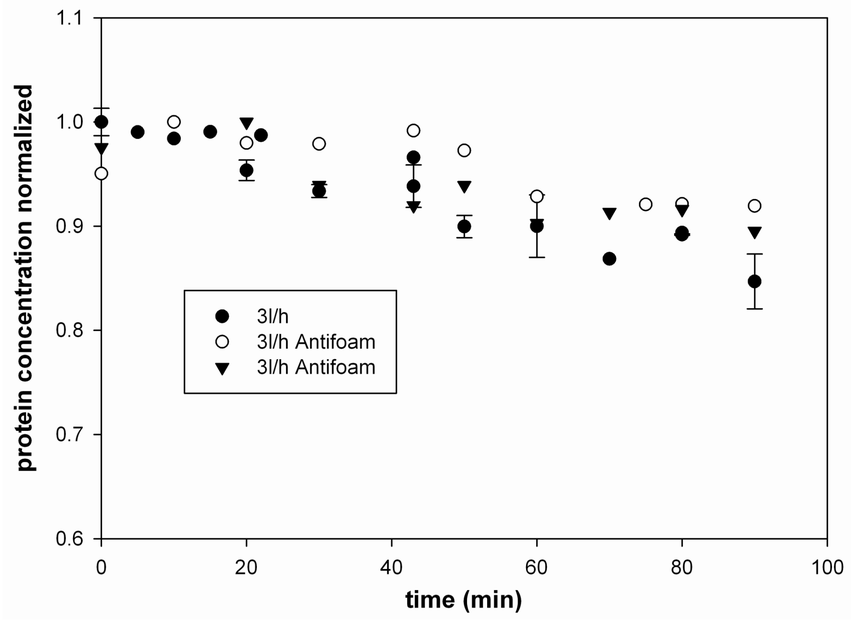
Figure 1: Protein loss under different aeration rates over time.

It was found that the loss of protein is dependent on the applied flow rate. Prior to kinetic analysis other essential effects were studied.

Foam formation

Figure 3a: Aeration of hGH (11.4 mL) with Antifoam PPG and aeration with foam formation.

Figure 3b: Influence of foam formation on protein loss due to aggregation.



The foam formation in aeration experiments was rather strong. Therefore the effect of this additional interface was studied. Preliminary data show that even without strong foam formation hGH is sensitive to air/liquid interfaces.

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