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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Introduction

Immunogenicity of therapeutic proteins remains a major concern. For example, the appearance of antibodies against the drug can cause safety problems such as autoimmune disorders by neutralizing the endogenously secreted proteins. Protein aggregates present in pharmaceuticals are considered to be a critical attribute in terms of their potential to elicit immune response and affect the product activity. For this reason, protein aggregates should be minimized as much as possible. The aggregates were then characterized in detail and accurately quantified. The present studies have thus been conducted in order (i) to explore the optimal formulation to minimize aggregation, (ii) to characterize in detail the dimerized antibody and (iii) to measure the effect of conditions on quantification of protein aggregates by analytical ultracentrifugation-sedimantetion velocity (AUC-SV).

(i) Exploration of the optimal formulation to minimize aggregation

Since aggregation can occur during storage even if the aggregates were completely removed by thorough purification, it is very important to develop optimal formulation for minimizing aggregation. In the approach to optimal formulations, the melting temperature (T_m) has been used in some cases based on the fact that higher T_m generally results in decreased aggregation, whereas the reversibility of thermally-induced denaturation has been used in other cases. Thus, which parameter that provides for optimal formulations depends on the property of the system. To investigate the reason of the different physical parameters that optimize the formulation, we measured T_m and reversibility and also quantitated the heat-induced aggregation of α-1-acid glycoprotein (AGP) in aqueous solutions at various pH values. It was shown that reduced conformational stability caused the formation of large aggregates via non-covalent association, whereas reduced reversibility was related to dimerization. This appears to indicate that conformational stability and reversibility are related to the formation of different kinds of protein aggregates. Thus, evaluating both parameters, T_m and reversibility, is important to develop optimal formulations for minimizing aggregation. The present results are useful not only for exploring optimal formulation, but also, e.g., for investigating the cause of unusual aggregates or unexpected increase of aggregates.

(ii) Detailed characterization of dimerized antibody

When trace amount of aggregated protein is generated upon storage, the aggregated protein needs to be characterized in detail in order to understand the effect of aggregation on the safety and efficacy. As antibody dimer species is often encountered, we focused on the dimer species in palivizumab, a humanized monoclonal antibody (IgG1). In order

to characterize the physical and chemical properties of the dimerized antibody, the intermolecular interactions, conformations and antigen binding properties of the dimer were investigated. The results indicated that approximately half of the dimer species was non-covalently associated, whereas the other half was dimerized by covalent bond including disulfide and di-tyrosine bonds. The dimer species were formed between F_{ab} and F_c or F_{ab} and F_{ab}. The higher-order structure and thermal stability of dimer and monomer were very similar, and the dimer retained the antigen binding activity as expected. The present study indicated that although major features of dimerization appear to be common among the IgG1 antibodies based on the previously reported results, the details were found to vary. The present data on the covalent dimer formation will be valuable in manufacturing process establishment to minimize dimer formation.

(iii) Effect of conditions on the measurement precision of aggregates by AUC-SV

In the minimization and characterization of protein aggregates, SEC is frequently used to measure the amount of aggregates and to collect the aggregated species. However, the capacity of the SEC to measure the aggregates should be checked by the distinct method. AUC-SV has been widely used to check the capacity based on the fact that it does not utilize the carrier or the gel. Although the source of variability in AUC-SV measurement became clearer owing to recent studies, the precision of AUC-SV is still lower than that of SEC. In order to search for unknown factors which could lower the precision, we investigated the effect of conditions of measurement such as rotor temperature, rotor speed and components of solvents. Although previously unknown factor was not found in the present study, the conventional rotor temperature and speed turned out to be appropriate. Also, sorbitol was shown to affect the *s*-value of the protein in concentration-dependent manner, probably through protein hydration. The stabilizing effect of sorbitol on the pharmaceutical protein could be estimated based on the sorbitol concentration- dependence of the *s*-value.