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論文内容の要旨

Abstract

In this study, biophysical characterization of a number of commercial pharmaceutical antibodies was performed by using size-exclusion chromatography (SEC) and analytical ultracentrifugation sedimentation velocity (AUC-SV). The AUC-SV experiments conducted at different rotational speeds revealed that antibody conformation can be accurately measured at 20,000 rpm. To account for the observed dependence of antibody hydration on rotational speed, it is suggested that correction factors should be introduced to the equation describing the process of sedimentation. A standard operating procedure for the precise quantification of aggregates in immunoglobulin preparations by using AUC-SV was designed. Following the developed procedure, it was shown that AUC-SV can be effectively applied to support the results obtained by SEC. Finally, by using AUC-SV, it was demonstrated that the percentages of higher order aggregates measured by the SEC method specifically designed for the regulation purposes do not represent the actual values. The findings of this study can have many important applications in therapeutic antibody research, such as in investigations of the effect of various formulations conditions on the stability of antibodies.

Chapter 1. General Introduction

Upon exposure to a variety of stresses encountered during manufacturing, pharmaceutical antibodies can undergo conformational changes. Induced conformational changes affect antibody stability and activity. Moreover, perturbations of antibody native structure may lead to the formation of aggregates, which cause loss of the therapeutic efficacy and induce allergic reactions. Therefore, it is essential to perform biophysical characterization of pharmaceutical antibodies to ensure safety and quality of antibody drug products. Several analytical techniques can be employed for the biophysical characterization of pharmaceutical samples, including

size-exclusion chromatography (SEC) and analytical ultracentrifugation sedimentation velocity (AUC-SV). The objectives of this study are to evaluate the conformational stability of antibodies by using AUC-SV and to assess the level of antibody aggregates present in the formulations by using AUC-SV to cross-validate the SEC results.

Chapter 2. Effects of rotational speed on the hydrodynamic properties of pharmaceutical antibodies measured by analytical ultracentrifugation sedimentation velocity (AUC-SV)

In general, the measured by AUC-SV hydrodynamic parameters are assumed to be speed-independent. For this reason, the influence of rotational speed on the hydrodynamic properties of antibodies has not been investigated. The objective of the present chapter is to investigate the effect of rotational speed on the hydrodynamic parameters of antibodies for the conformational analysis. Two antibodies were studied by AUC-SV at five different rotor speeds, and acquired data were analyzed using SEDFIT and UltraScan computer programs. The measured frictional ratio and molecular weight were inversely proportional to rotational speed and the most accurate estimations of both parameters were achieved at 20,000 rpm. It was confirmed that antibody adopted conformation similar to that revealed by X-ray crystallographic analysis. These findings clearly demonstrate that AUC-SV is a powerful tool for the accurate conformational analysis of pharmaceutical antibodies.

Chapter 3. Aggregation analysis of pharmaceutical human immunoglobulin preparations using size-exclusion chromatography (SEC) and analytical ultracentrifugation sedimentation velocity (AUC-SV)

Due to a nonspecific binding of protein aggregates to packing material of the SEC column, which was previously reported, the results of SEC should be verified using different analytical techniques. The objective of this chapter is to demonstrate that AUC-SV can effectively be used for the quantification of aggregates in pharmaceutical immunoglobulin preparations and to confirm the performance of SEC. For this purpose, a standard operating procedure for the measurements of aggregates using AUC-SV was developed. Following the procedure, seven formulations, four liquid and three lyophilized, were studied by using AUC-SV and obtained results were compared with those of SEC under standard conditions. AUC-SV and SEC provided similar results of quantification of aggregates. Thus, it is demonstrated that AUC-SV can be effectively used for the aggregation analysis of immunoglobulin preparations and to confirm the performance of SEC.

Chapter 4. Role of analytical ultracentrifugation sedimentation velocity (AUC-SV) in the regulation of biopharmaceutical drugs

To ensure performance of the optimized SEC method, it is important to evaluate its results using alternative methods. The objective of this chapter is to confirm the performance of the SEC method specifically designed for regulation purposes by using AUC-SV. Seven pharmaceutical antibodies formulations, four liquid and three lyophilized, were studied by using AUC-SV and SEC using optimized elution conditions. The amounts of dimeric and higher order aggregates determined by the SEC analysis were higher than those estimated by AUC-SV. The SEC method with optimized conditions provided estimation of higher order aggregates of more than 1.0%. However, AUC-SV analysis revealed that the actual value was less than 1.0%.

Chapter 5. General conclusion

AUC-SV is a powerful tool for the comprehensive biophysical characterization of pharmaceutical antibodies. It can yield useful information regarding conformational stability of antibody in various formulations used during manufacturing steps. AUC-SV provides higher resolution than SEC, particularly for high-molecular weight aggregates. By using AUC-SV, it is possible to assess whether the optimized SEC method accurately quantifies aggregates.

List of publications

1. Krayukhina, E., Uchiyama, S., Fukui, K., 2012a. Effects of rotational speed on the hydrodynamic properties of pharmaceutical antibodies measured by analytical ultracentrifugation sedimentation velocity. Eur. J. Pharm. Sci. 47, 367-374.

2. Krayukhina, E., Uchiyama, S., Nojima, K., Okada, Y., Hamaguchi, I., Fukui, K., 2012b. Aggregation analysis of pharmaceutical human immunoglobulin preparations using size-exclusion chromatography and analytical ultracentrifugation sedimentation velocity. J. Biosci. Bioeng., doi: 10.1016/j.jbiosc.2012.07.021

3. Nojima, K., Krayukhina, E., Uchiyama, S., Fukui, K., Okada, Y., Hamaguchi, I., 2012. Appropriate evaluation of globulin aggregates and oligomers in human plasma derived intravenous immunoglobulin (in preparation, to be submitted to Vox Sang.).

4. Krayukhina, E., Iwasaki, H., Nojima, K., Uchiyama, S., Okada, Y., Hamaguchi, I., Fukui, K. Quantification of antibody aggregates by high-performance size-exclusion chromatography and ultra-performance liquid chromatography (in preparation).

論文審査の結果の要旨

本論文は、タンパク質の溶液中でのコンフォメーションと分散状態の定量的解析を実現する超遠心分析沈降速度法（沈降速度法）を確立し、実際に抗体医薬やイムノグロブリン製剤中の抗体のコンフォメーションと凝集体含量を解析することで、沈降速度法の有効性を示している。

第一章は、抗体医薬の物理化学分析は製造コストのみならず副作用の観点からも不可欠である。そこで CPU の計算速度の向上により数値解析を用いた沈降データの直接解析が可能となった超遠心分析沈降速度法（沈降速度法）が注目を浴びつつあることが述べられている。

第二章は、沈降速度法でコンフォメーション解析を行う際には、適切な回転速度で測定を行う必要があることを述べている。とくに 20,000rpm で測定を行えば沈降速度法によりコンフォメーションを正確に評価可能であることが述べており、実際、抗体について沈降速度法により摩擦係数（実測値）を求めたところ、結晶構造解析により得られている三次元座標を基にビーズモデリング法により求めた摩擦係数（計算値）と良く一致しており、溶液中で結晶構造と類似のコンフォメーションをとっていることが示されている。

第三章は、沈降速度法の測定および解析手順の最適化を行い、高精度で再現性良く凝集体含量を定量可能な手順を確立した後、確立した手法により市販の 7 種類のイムノグロブリン製剤について PBS を用いて解析を行った結果を示している。その結果、全ての製剤について SEC で求めた凝集体含量は沈降速度法より求めた値よりも大きくなっており、現行の基準値について検討の余地があることが示されている。

最後に得られた知見を総括しバイオ医薬分析における沈降速度法の有効性と将来展望について記述している。

本論文は、バイオ医薬品の溶液中でのコンフォメーション解析と凝集体定量を沈降速度法により実現する測定条件を見出し、沈降速度法が医薬品開発および品質管理における要請に十分に応えられる手法であることを示している。また、SEC 法による凝集体定量はピークの分離方法によって異なる凝集体含量となる等の課題を内包しており、沈降速度法による結果のバリデーションが必要であることを示している。これらの結果はバイオ医薬品開発における沈降速度法の有用性を示すものであり、生物工学、医薬品の化学工学の分野で貢献するところが大きい。

よって本論文は博士論文として価値あるものと認める。