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論　文　内　容　の　要　旨

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論文題名	Identification of Modifications in Pharmaceutical Antibodies by Mass Spectrometry （質量分析による抗体医薬品の修飾体解析）

論文内容の要旨

Chapter 1: General Introduction

Pharmaceutical antibodies are medicinal products which contain antibodies as active pharmaceutical ingredients. The market of pharmaceutical antibodies is still growing and a lot of extensive pipeline of candidates for pharmaceutical antibodies are on their way of human clinical trials.
Characterization of pharmaceutical antibodies is one of the important issues in the development of pharmaceutical antibodies to provide effective and safe drug products to the patients. Mass spectrometry is a powerful tool to characterize the antibodies especially for the primary structure analysis. In this study, an analytical platform was developed for the primary structure analysis of pharmaceutical antibodies by improving the state-of-art techniques of mass spectrometry.

Chapter 2: Methodology for the Primary Structure Analysis of IgG

Although mass spectrometry techniques had been rapidly maturing with the growth of the pharmaceutical antibody market over the past decades, there were still some problems to be solved. In this chapter, we investigated four techniques to overcome such problems. At first, mass errors in the intact mass was achieved to decrease by the middle down approach, a specific digestion at the hinge region of antibodies, deglycosylation and reduction. On top of that, isobaric modifications were elucidated by the forced degradation studies with isotopic labeling. And then, modifications could be quantitated accurately enough by the target based approach, preparing the XIC of the peptide fragments including the target modification. Finally, structures of unknown chemical modifications were revealed by de novo sequencing and amino acid analyses.

Chapter 3: Identification of a Racemized Cysteine Residue in the Hinge Region of IgG1

The hinge region of IgG1 molecules are highly susceptible to be modified. For example, Asp221 was found to undergo isomerization and also ladder cleavage was observed in the hinge region. Potential modifications in the hinge region can be suppressed effectively by elucidating the reaction pathways and establishing a strictly controlled storage condition. In this chapter, the specific racemization of cysteine was identified as a novel modification in the hinge region by mass spectrometry. We demonstrated a reaction mechanism involving a base catalysis, which gave us a clue to control the modifications in the hinge region.

Chapter 4: Identification of Histidine Oxidation in a Pharmaceutical Antibody

Pharmaceutical antibodies are exposed to light during manufacturing, packaging, and using. The light stress induce oxidations of antibodies: Oxidation of methionine affects efficacy and pharmaco kinetics, and oxidation of Trp in the CDR regions affects efficacy. Though oxidation of histidine had been reported in other proteins, it had not been reported in the antibodies. In this chapter, oxidation of histidine was identified at CH2 domain as a novel modification when IgG1 was exposed to light. By clarifying the reaction pathway of the oxidation, it was considered that this reaction could be suppressed by the decrease of the dissolved oxygen.

Chapter 5: General Conclusion

We developed techniques for the primary structure analysis of pharmaceutical antibodies by mass spectrometry.

Using the techniques, racemization of cysteine in the hinge region was identified, which gave a clue to control the modifications in the hinge region. Oxidation of histidine in the CH2 domain was identified and it was suggested that dissolved oxygen was a key to control the oxidation. Revealing novel modification sites provides the possibilities to characterize antibodies more strictly and easily in the near future. In addition, primary structure analysis by mass spectrometry is very powerful method for the primary structure analysis of not only antibodies but also other proteins.

Appendix: Control of Methionine Oxidations by Container Closure Systems

To control oxidation of pharmaceutical antibodies, potential sequences for the oxidation should not be employed and optimal formulation conditions should be selected. However, it was difficult to completely suppress the oxidations. Therefore, we investigated container and closure systems, plastic syringe, and glass syringe with or without oxygen absorber to control the oxidation levels.

The lowest methionine oxidation level was observed for a plastic syringe with oxygen absorber. The oxidation levels were likely to be related to the concentrations of dissolved oxygen. Acidic variants, high molecular weight species, and low molecular weight species could also be controlled by regulating dissolved oxygen; however, the oxidative species in them were still unknown.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

抗体医薬品は、優れた薬効および安全性が期待できる次世代医薬品として現在、数多くのものの開発が進められている。患者にとってより安全かつ有効な抗体医薬品を開発するために抗体医薬の特性解析が重視されており、質量分析法は一次構造解析のための有効な手段として認識されている。このような状況に基づき、学位申請者は、最新の質量分析技術を駆使して、抗体医薬品の一次構造の解析のために必要な新しい分析プラットフォームを開発する。

近年、質量分析法の進展には著しいものがあるが、一次構造解析には現在でもいくつかの問題が残されている。そこで、そうした問題を解決するために４つのアプローチを用いるのである。具体的には、質量精度の上昇、既知の化学修飾の網羅的同定と正確な定量、未知の化学修飾の解明等々によるものである。

これらを用いて、IgG1 分子のヒンジ領域におけるシスティン残基の化学修飾の同定を試みるのである。何故なら IgG1 分子のヒンジ領域は極めて容易に変異するのである。本研究では従来知られていなかったシスティン残基のラセミ化を、塩基触媒機構によると考えられるその反応機構を含めて、質量分析法を用いて明らかにすることに成功している。これは将来、ラセミ化を制御することにもつながる発見である。

最後に抗体医薬品におけるヒスチジンの酸化についても検討している。酸化は抗体医薬の生産、パッケージング、使用の全ての段階で光の影響により生じ、抗体医薬の効果に影響を与えることが知られている。ヒスチジンの酸化は従来は抗体では知られていなかったが、本研究により、IgG1 が光に照射されることにより、CH2 領域で生じることが明らかにされる。かつ、その反応機構から溶存酸素を減少させることにより、この反応を抑制できる見通しが立つのである。

以上のように、本論文は、新しい質量分析プラットフォームを用いて抗体医薬品の一次構造における種々の変異や修飾を解明するものであり、その成果は抗体のみならず種々のタンパク質の一次構造の解析に広く利用できるものである。よって本論文は博士論文として価値あるものと認める。