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Application News

No. B63A

Protein Sequencer

N-Terminal Amino Acid Sequencing of IgG Antibodies

Foreword

Recently, the term "biomedicine" is often heard in the field of pharmaceuticals. While also called biopharmaceuticals, they refer to proteinaceous drugs and antibody drugs developed and manufactured using biotechnologies including genetic recombination, cell fusion, and cell culture. In contrast, conventional medicines are referred to as "low molecular drugs" and are produced through chemical synthesis. While both types are chemical compounds, compared to chemical synthesized low molecular drugs, biomedicines characteristically have a much higher molecular weight. Of the top 10 drugs in all pharmaceuticals sold worldwide in 2015, seven were biomedicines.

Biomedicines are highly effective, low in side effects, and can be used to treat a wide range of illnesses. Unfortunately, unlike low molecular drugs that until now have been mainstream, mass production of biomedicines is not possible in the same way as chemical synthesized products. Biomedicine production comprises multiple processes including manufacture, refinement, dosage form design, and storage. In order to guarantee the quality of biomedicines, influences originating from raw materials and manufacturing processes need to be taken into consideration in addition to performing qualification testing of products. This means that management of manufacturing and quality of drugs requires a different approach compared to chemical synthesized low molecular drug products. Guidelines currently exist for evaluating the quality of biomedicines. These guidelines require that characteristic analysis is performed and one type of characteristic analysis is N-terminal amino acid sequencing. This analysis is performed to compare and verify the N-terminal amino acid sequence presumed from the gene sequence with the N-terminal amino acid sequence of the biomedicine product. The analysis technique used is the Edman method. This technique determines amino sequences by cleaving amino acids sequentially from the N-terminus of proteins and obtains very reliable amino acid sequences. The PPSQ™-51A/53A Protein Sequencer is a system that automates this technique. This system facilitates identification of amino sequences from the N-terminus of target proteins and peptides.

This article introduces an example of amino acid sequencing of mouse antibody IgG using the PPSQ-51A/53A Protein Sequencer isocratic system as an instance of N-terminal amino acid sequencing of biomedicines.

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Method

The basic structure of antibodies comprises two H chains (heavy chains with higher molecular weight) and two L chains (light chains with lower molecular weight).
Since intact antibodies have high molecular weight (approx. 150 kDa), performing N-terminal amino acid sequencing using the Protein Sequencer in this state proves to be very difficult. In this analysis example, 10 pmol of mouse monoclonal antibody IgG was separated into H chains and L chains by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and these chains were then electroblotted onto a PVDF membrane. The PVDF membranes of CBB-stained L chains and H chains were analyzed using the PPSQ-53A Protein Sequencer isocratic system.

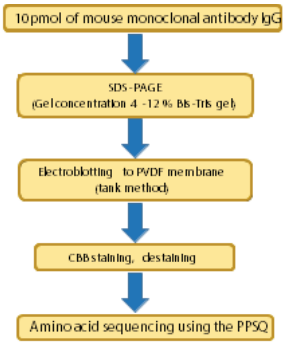

Fig. 1 Protocol for N-Terminal Amino Acid Sequencing

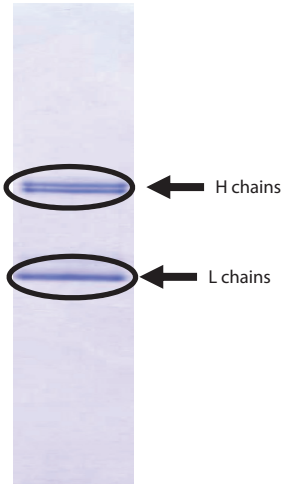

Fig. 2 PVDF Membrane After Electroblotting

Results of N-Terminal Amino Acid Sequencing

Fig. 3 and Fig. 4 show the results of N-terminal amino acid sequencing of L chains and H chains from the mouse monoclonal antibody IgG (SIGMA-ALDRICH cat#I5381). Each figure shows chromatograms from cycle 1 to 5 for each sample (cycle 1 is the raw chromatogram and others are subtracted chromatograms). In Fig. 3, cycle 1 in the L-chain results identified the amino acid residue of the N-terminus as asparagine (Asp) and cycle 2 identified the second amino acid residue of the N-terminus as isoleucine (Ile). After performing analysis to the 21th residue, the sequence from the N-terminus was identified as Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ala-Ser-Leu-Ser-Ala(Val)-Ser-Val-Gly-Glu-Thr-Val-Thr-Ile. Searching the database revealed this to be kappa light chain IgG1.

Likewise, Fig. 4 shows the H-chain results up to cycle 5. The sequence from the N-terminus was identified as Glu-Val-Gln-Leu-Gln-Glu-Ser-Gly-Pro-Glu-Leu-Val-Lys-Pro-Gly-Ala-Ser and a database search revealed this to be immunoglobulin alpha heavy chain. As shown in this example, performing analysis with the Protein Sequencer enables easy and accurate identification of N-terminal sequences and demonstrates that the Protein Sequencer is an effective system for managing the quality of biomedicines.

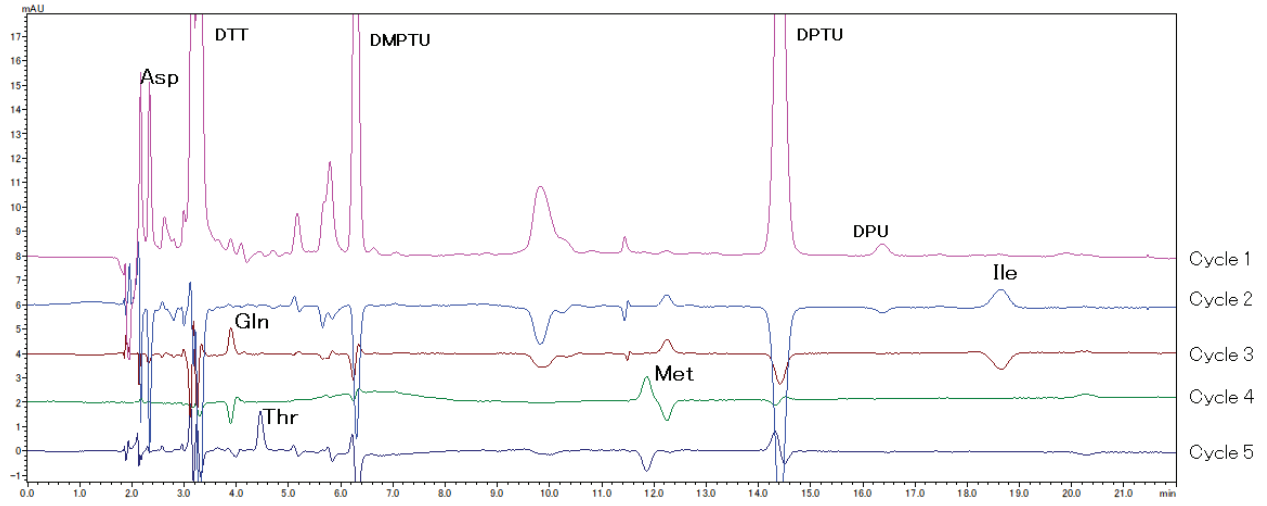

Fig. 3 L-Chain Chromatograms (Cycles 1 to 5)

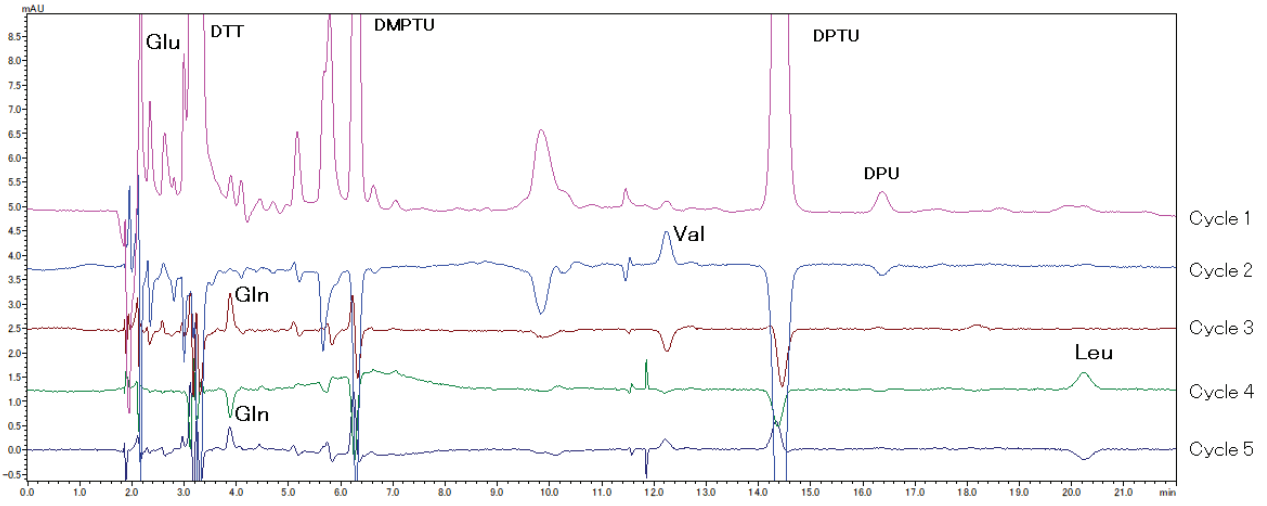

Fig. 4 H-Chain Chromatograms (Cycles 1 to 5)

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