Mass spectrometry sequencing of a SARS-CoV-2 anti-spike S1 monoclonal antibody

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Introduction: In 2020, the world faced the SARS-CoV-2 (Severe Acute Respiratory Syndrome 2) virus, prompting the World Health Organization to declare a global health emergency, which lasted three years. Vaccines remain the most effective preventive measure against COVID-19 (Coronavirus disease 19), as they continue to promote mass immunization against new strains of the virus and contain new waves of infection. However, many immunocompromised patients show a weaker response to vaccination, reducing its efficacy against SARS-CoV-2. An alternative for these individuals is monoclonal antibody therapy. Immunotherapy and prevention with monoclonal antibodies have already been used in other viral infections, such as RSV, MERS-CoV, and Ebola. For COVID-19, although some monoclonal antibodies were approved for treating severe cases, the emergence of new variants has reduced their neutralization efficacy. Hence, developing a platform that enables rapid antibody sequencing would potentially identify neutralizing antibodies against new variants and keep the antibody-based therapy updated. As a proof-of-concept for a rapid sequencing methodology, we sequenced the light and heavy chains of a commercial SARS-CoV-2 anti-spike S1 monoclonal antibody. **Materials and methods:** the monoclonal antibody Anti-SARS-CoV-2 spike S1, code 0872030-CF from MPBio, whose sequence was unknown, was analyzed in this project. Monoclonal antibody samples were reduced, alkylated, aliquoted, and separately digested with five different enzymes: trypsin, chymotrypsin, Asp-N, Glu-C, and thermolysin. The antibody heavy and light chains were cleaved at different sites and subsequently desalted with C18 tips. LC-MS/MS analysis was performed on an Orbitrap Exploris 240 (Thermo Scientific) coupled to an Easy-nLC 1200 (Proxeon Biosystem, USA). The mass spectrometer was operated in data-dependent acquisition mode at 20 MS/MS scans/cycle. For database search, *Mus musculus* immunoglobulin databases from Uniprot and IMGT were used. Sequencing of the variable regions was performed by manual evaluation of *de novo* overlapping MS/MS spectra and iterative database search in PEAKS Studio X+ (Bioinformatics Solutions Inc.). **Results and discussion:** using five LC-MS/MS runs, one for each enzyme, and HCD fragmentation we completely sequenced the heavy and light chains of the SARS-CoV-2 anti-spike S1 monoclonal antibody. The sequence information of the heavy and light chains will be used to express the monoclonal antibody in HEK293T cells. The expressed IgG will be further evaluated by Western blot, to compare its activity with the original commercial antibody. **Conclusion:** we successfully used a mass spectrometry-based workflow to completely sequence the heavy and light chains of a SARS-CoV-2 anti-spike S1 monoclonal antibody. The methodology can be applied to other antibodies and proteins of non-model organisms.

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