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Mass Spectrometry and COVID-19: Characterization and Diagnostics

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Abstract

The emergence of SARS-CoV-2 (disease:COVID-19) has created a severe public health crisis with an ongoing pandemic and according to the World Health Organization, over 2.5 million confirmed cases of infection to date. Analytical science and techniques are at the forefront of ongoing research and development and ranges from understanding the fundamental biology of the virus to diagnostic testing. This editorial examines mass spectrometry based analytical strategies used in virus characterization and diagnostics; a summary of what has been done and what is emerging and evolving is presented.

Keywords: COVID-19, Mass spectrometry, diagnostics, characterization, Spike Protein, SARS-CoV-2.

Short communication

Established methods in mass spectrometry (MS) used in the characterization of virus structure and, protein-based vaccines and therapeutics which target viral infections include peptide mapping [1], glycan profiling (site localization, composition and heterogeneity) [2] and quantification of viral proteins with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [3]. These methods usually involve digestion of proteins (free or incorporated into virus, vaccine or therapeutic assembly/structure) into peptides prior to MS analysis; glycan analysis can be done at the glycopeptide level or glycans can be released and oftentimes labeled for ease of ionization prior to MS analysis or allow for detection with complementary methods[4,5].

In biopharmaceutical research and development peptide mapping is a first step in the assessment of the critical quality attributes (CQAs) [6] of a protein-based product. LC-MS/MS peptide mapping assay serve to confirm the primary sequence of a protein as proof of identity. Glycans can affect safety, stability, efficacy and clearance of a biotherapeutic and so its glycan profile is also a CQA [7]. In the ongoing pandemic, virions of SARS-CoV-2 (the virus which causes COVID-19) comprise single-stranded RNA with nucleoproteins enclosed within a capsid containing matrix proteins; four structural proteins are present-S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins [8,9]. The N protein encapsulates the RNA genome while the S, E, and M proteins make up the viral envelope. The S protein, which protrudes from the virus surface giving it a crown-like appearance, is the subject of extensive characterization as it is a key target for vaccine and drug development because it initiates and mediates entry into the host cell and subsequent infection [10]. It is a ~540 kDa homotrimer complex heterogeneously glycosylated with 22 N-glycan sites on each monomer. Peptide mapping has been used to obtain the primary sequence of recombinant SARS-CoV-2 Spike Protein at about 90% sequence coverage [11]. The glycans on the S-Protein surface can play several roles including that of immune evasion by forming a glycan shield, host cell receptor binding and im- mune recognition [12,13]. Thus, for effective drug design and to aid in
understanding virus action and infection a glycan profile is vital. The N-glycan site localization and composition has been determined with LC-MS/MS for all 22 potential glycosylation sites on the native S protein allowing for a signature N-glycan profile [14]. In addition, LC-MS/MS was used to experimentally deduce for the first time O-glycans on the S-Protein surface [15].

Most mass spectrometry based diagnostic assays for COVID-19 (to date) use a targeted LC-MS/MS proteomics approach with multiple reaction monitoring (MRM) [16]. Currently the main diagnostic test for COVID-19 is based upon the real time polymerase chain reaction (rT-PCR) and detects specific viral signatures of the SARS-CoV-2 genome in people who are infected. It is the gold standard for testing, but it has limitations and includes false positives, low detection sensitivity and reduced specificity particularly in a mutated virus [17]. Antibody based serology COVID-19 tests can be used to detect viral antigens, but the limitation arises with a negative (inconclusive) result since the detection sensitivity and specificity can be suboptimal [18,19]. Proteomics based testing would serve as a complementary, fast, sensitive and specific method in COVID-19 diagnostics. Targeted LC-MS/MS with MRM have been used for the detection of the SARS-CoV-2 via detection of signature peptides of the S and N-proteins. This approach has been successfully applied for the detection of the S Protein from nasooropharyngeal swabs in symptomatic and asymptomatic COVID-19 patients [20] and in Gargle solution samples from COVID-19 patients [21].

Although they have been applied to virus structure characterization with instrument modification, intact mass analysis, native mass spectrometry and hydrogen deuterium exchange (HDX) methods present more of a challenge because these megadalton sized assemblies are simply too large for conventional and commercial mass spectrometers currently available [22-25]. Characterization usually relies on full or partial digestion for MS analysis; however, information on intact assembly, antigenic protein incorporation into assembly and aggregation state can be lost. However, with the advent of the new charge detection mass spectrometry (CDMS) the upper mass limitation is overcome. CDMS allows the direct measurement of mass (allowing for confirmation of size, identity and confirmation of antigen incorporation into viral capsids or a drug product), mass distribution and aggregation state of megadalton sized structure [26-29]. Thus, in the biopharmaceutical world, it holds potential as an emerging and promising technique for the characterization of megadalton sized structures such as gene therapy vectors, viruses and vaccine candidates such as virus-like particles (VLPs) where confirmation of assembly structure, identity and assembly/disassembly may become routine with MS.

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Overall, established MS workflows have risen to the need for methods to be used in SARS-CoV-2 characterization. MS based diagnostic assays look promising, are still emerging and being developed. CDMS currently presents at the forefront of MS technologies for the characterization of megadalton sized structures at the fully assembled level; however, there is a need for the development of more techniques in this area.

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