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Application Note

Comprehending COVID-19: Reversed-Phase Liquid Chromatography (RPLC) of Intact SARS-CoV-2 Spike Protein

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The global COVID-19 pandemic has resulted in extensive efforts to develop vaccines for the novel coronavirus. Identifying vaccine targets relies on robust analytical methods to understand SARS-CoV-2 structural biology. This study focuses on reversed-phase liquid chromatographic analysis of the intact SARS-CoV-2 spike protein, which has emerged as a potential target for vaccine development due to its role in viral pathogenesis.[1,2] This work demonstrates that using difluoroacetic acid (DFA) as a mobile phase modifier in place of formic acid (FA) results in increased chromatographic resolution during intact protein analysis. Furthermore, the results suggest that pairing this approach with N- and O-glycosidase treatments may enable more detailed intact protein MS investigations.

Benefits

Using DFA instead of FA as the mobile phase modifier achieves:

- Higher resolution of less abundant proteoforms
- Three-fold increase in gradient peak capacity

Introduction

The SARS-CoV-2 spike protein, which facilitates host cell infection, has become a subject of detailed study due to its potential as a COVID-19 vaccine target. Proper characterization of this novel coronavirus protein relies on robust identity and purity tests. While extensive characterization work is underway to study the SARS-CoV-2 spike protein's glycans and glycopeptides, intact protein analysis using reversed-phase liquid chromatography (RPLC), either with or without the combined use of endoglycosidases, may offer unique analytical insights.[3,4]

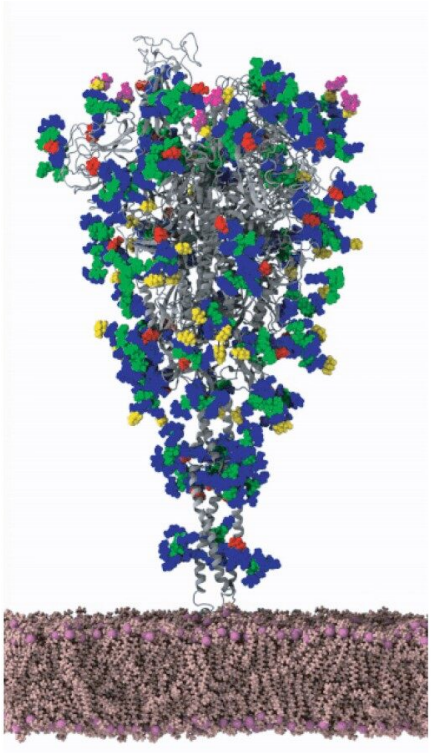

Figure 1. The SARS-CoV-2 spike protein (gray) with glycans modeled on its surface. Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego.

To aid this effort, Waters shares the following method:

- A comparison of an intact RPLC profile using mobile phases modified with either difluoroacetic acid (DFA) or formic acid (FA). DFA is shown to enhance resolving power while maintaining MS-compatibility.

Experimental

The following experimental conditions were used for RPLC-FLR-MS intact protein analysis of the SARS-CoV-2 spike protein.

LC Conditions

LC system:	ACQUITY UPLC I-Class
Detection:	FLR (280 nm emission, 320 nm excitation)
Vials:	QuanRecovery vials
Column(s):	BioResolve RP mAb Polyphenyl, 2.7 µm, 450 Å, 2.1 x 50 mm
Column temp.:	80 °C
Sample temp.:	8 °C
Injection volume:	1 µL
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% IonHance DFA or FA in water
Mobile phase B:	0.1% IonHance DFA or FA in acetonitrile
Gradient:	15–55% Mobile phase B in 20 minutes

MS Conditions

MS system:	Vion IMS QToF Mass Spectrometer
Ionization mode:	ESI+
Acquisition range:	1500–4000 *m/z*
Capillary voltage:	2.25 kV
Collision energy:	6 V

Cone voltage:	140 V

Results and Discussion

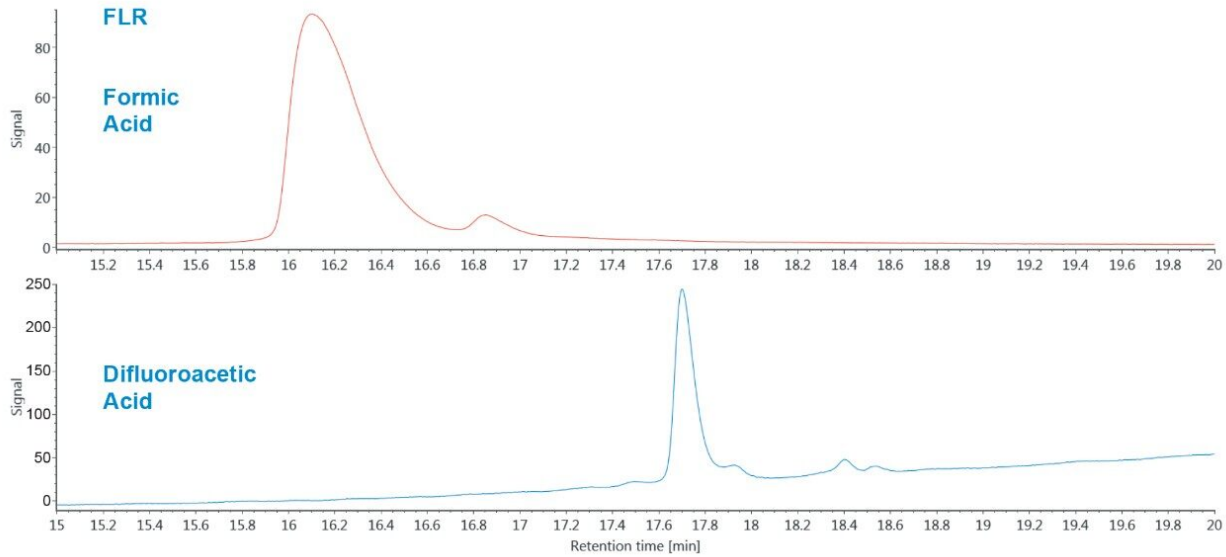

Figure 2. Comparison of FA and DFA Intact Protein Fluorescence Signal.

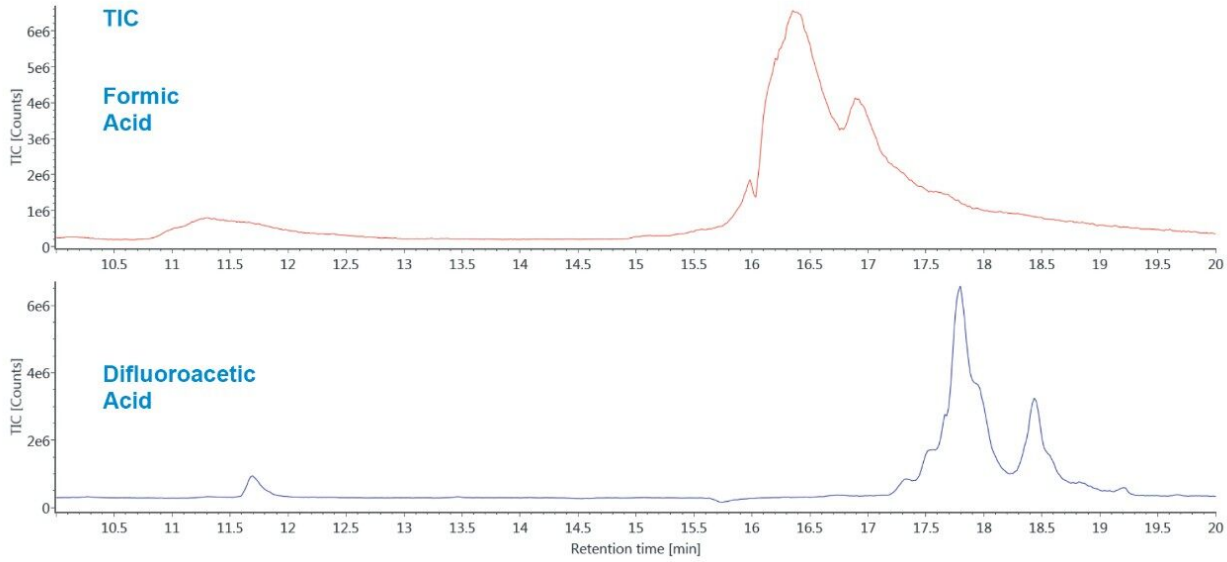

Figure 3. Comparison of FA and DFA Intact Protein Total Ion Chromatograms.

Employing DFA as a mobile phase modifier resulted in a comparatively higher resolution chromatogram. Compared to using FA as a mobile phase modifier, gradient peak capacity increased by over three-fold while the less abundant proteoforms were better resolved. Pairing this chromatographic approach with N- and O-glycosidase treatments may enable more detailed MS investigations at the intact protein level of analysis.

Conclusion

Because the SARS-CoV-2 spike protein is implicated in viral pathogenesis, it has become a target for vaccine development. Efficient therapeutic development relies on a solid structural and functional understanding of the SARS-CoV-2 spike protein target. Intact protein analysis using RPLC can be used to refine our understanding of the SARS-CoV-2 spike protein and thus help to identify and develop promising new COVID-19 therapies. This work demonstrates that the use of DFA instead of FA as mobile phase modifier enhances method resolving power while maintaining MS-compatibility.

References

1. Pinto, D. *et al*. Structural and Functional Analysis of a Potent Sarbecovirus Neutralizing Antibody. *bioRxiv* 2020.04.07.023903 (2020). doi: https://doi.org/10.1101/2020.04.07.023903 < https://doi.org/10.1101/2020.04.07.023903>

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3. Liu, X. and Lauber, M. Comprehending COVID-19: Rapid and Sensitive Characterization of N-Glycans from SARS-CoV-2 Spike Protein. Waters Application Highlight 720006914 < https://www.waters.com/nextgen/us/en/library/application-notes/2020/comprehending-covid-19-rapid-and-sensitive-characterization-of-n-glycans-from-sars-cov-2-spike-protein.html> .

4. Novokmet, Mislav *et al*. Understanding Glycans in COVID-19 Drug Design. https://www.genengnews.com/insights/understanding-glycans-in-covid-19-drug-design/ < https://www.genengnews.com/insights/understanding-glycans-in-covid-19-drug-design/>

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