MOLECULAR CHARACTERIZATION OF *GLOECAPSOMORPHA PRISCA* MICROFOSSILS BY MASS SPECTROMETRY DOWN TO THE CELLULAR SCALE

Bon M. [1,2,3], Lepot K. [1,4], Carpentier Y. [2], Bray F [5], Riboulleau A. [1], Baudin F. [6], Nuns N. [7], Rolando C. [5], Steemans P. [8] and Vandenbroucke T. R.A. [3]

[1]Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187 – LOG – Laboratoire d'Océanologie et de Géosciences, F-59000 Lille, France, [2]Univ. Lille, CNRS, UMR 8523 – PhLAM – Physique des Lasers Atomes et Molécules, F-59000 Lille, France, [3]Department of Geology (WE13), Ghent University, Ghent, 9000, Belgium, [4]Institut Universitaire de France (IUF), [5]Univ. Lille, CNRS, USR 3290 – MSAP – Miniaturisation pour la Synthèse, l'Analyse et la Protéomique, F-59000 Lille, France, [6]SU CNRS, ISTeP UMR 7193, 75005 Paris, France, [7]Univ. Lille, CNRS, Centrale Lille, Univ. Artois, UMR 8181 - UCCS - Unité de Catalyse et Chimie du Solide, F-59000 Lille, France, [8]EDDy Lab/Palaeobotany and Palaeopalynology, Univ. Liège, 4000 Liège, Belgium

Introduction

Gloeocapsomorpha prisca (Fig. 1) is a microfossil (~10µm) with an organic wall of uncertain biological affinity, probably a cyanobacterium[1,2,4,6]. This fossil microorganism constitutes the vast majority of the organic matter in "kukersite" rocks. *G. prisca* has been thoroughly studied by gas chromatography-mass spectrometry (GC-MS), using extractive techniques, chemolysis and pyrolysis[1,2,3]. Here we perform a pilot study in order to investigate the potential of laser-assisted mass spectrometry to analyze the molecular fingerprint of single microfossils. Bitumen and kerogen of a kukersite sample from a 460-million-year-old deposit of northwestern Russia[5], were analyzed after organic solvent extractions and demineralization. We measure bulk thermo-desorption and pyrolysis parameters with Rock-Eval 7S. We use molecular characterization methods that allow analyses down to the scale of a single microfossil. We analyze kerogen by secondary ion time-of-flight mass spectrometry (ToF-SIMS), which combines extreme surface sensitivity with a spatial resolution of ~1 µm. The kerogen is then analyzed with high resolution (HR-µL2-MS) two-step laser desorption-ionization mass spectrometry with a spatial resolution of 140 µm. We used very-high mass resolution Fourier transform ion cyclotron resonance mass spectrometry by laser desorption/ionization (LDI-FT-ICR-MS) on *G. prisca* aggregates (e.g., Fig. 1) with laser beams down to less than 20 µm.

Results

According to Rock-Eval the organic matter is immature, and the kerogen is type I with low percentage of organic sulfur. The ToF-SIMS analysis of microfossils shows small aliphatic fragments and carbonaceous clusters ($C_nH_{0-3}^+$) generated by fragmentation and abundant (poly)aromatic hydrocarbons, as well as a minor contribution of oxygenated and nitrogenated compounds. Using two-step laser desorption-ionization (HR-µL2-MS) we observe a relatively similar composition but with higher molecular weights. The spectra obtained by LDI-FT-ICR-MS show aromatic hydrocarbons, oxygenated and nitrogenous compounds, with a major contribution of the oxygenated compounds. This predominance is in agreement with the composition of *G. prisca* models based on chemolysis-assisted GC-MS and pyrolysis GC-MS[1,2,3,4]. LDI-FT-ICR-MS allowed us to characterize the bulk kerogen (spectrum in Fig. 1) as well as a single aggregate of microfossils. Unambiguous formula assignment could be carried for ions with m/z up to 400 or more in LDI-FT-ICR-MS, which helps us to represent the molecular compositions of ions generated by laser-desorption ionization in van Krevelen plots (O/C vs H/C) and mass-defect plots. Potential effects of the desorption lasers, such as fragmentation and pyrolysis, can be discussed by comparison of the data generated at various laser fluences and spot sizes. Similarly, the composition of the bitumen is distinguished from that of solvent-extracted microfossils.

Conclusions

The three mass spectrometry techniques each have their specificities. The apparent aromaticity observed could be related to the pyrolysis in LDI and/or to the selectivity of the UV ionization, in particular for the post-desorption ionization at 266 nm in L2-MS and to the fragmentation of the aliphatic chains in SIMS. Nevertheless, the predominance of oxygenated compounds (O_2, O_3, O_4) seen in LDI-FT-ICR-MS (Fig. 1), including some high H/C ions (e.g., $C_{12}H_{17}O_2^+$), is consistent with fragmentation, possibly associated with rearrangements and/or pyrolysis, of the *n*-alkylresorcinol based biopolymer proposed for the composition of *G. prisca*[1].

This approach will be applied to the search for possible chemotaxonomic parameters of different morphospecies from other samples with a more diverse microfossil assemblage. Our protocols have potential to become benchmarks in the search for molecular signatures for exobiology, as, for example, an LDI-MS instrument has been developed for the ExoMars rover[7].

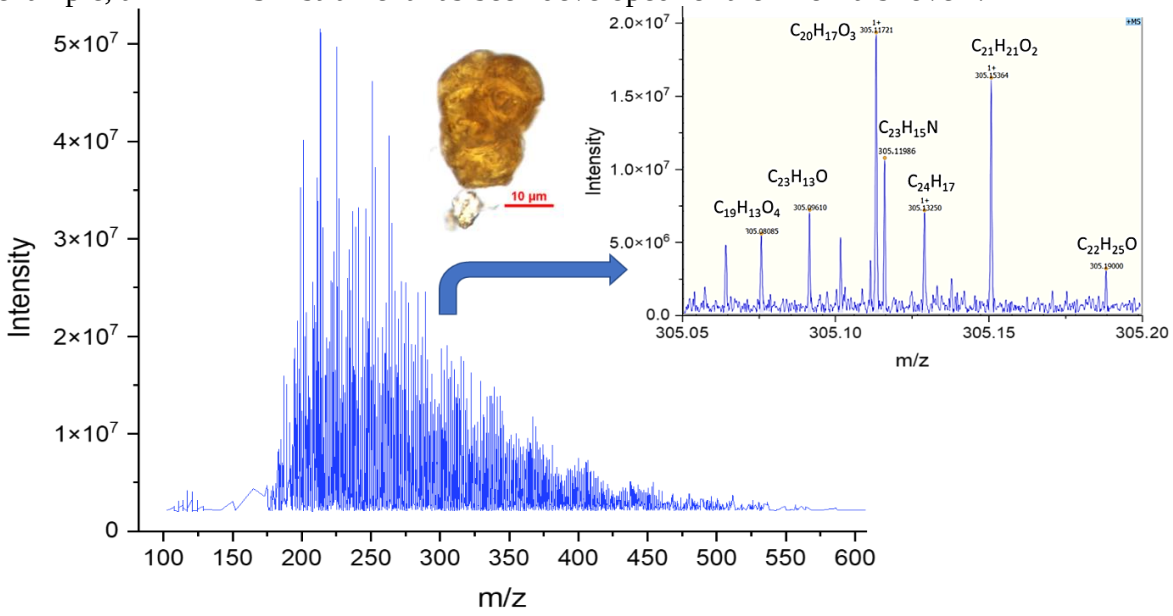

***Figure 1**: total spectrum of bulk* G. prisca *LDI-FT-ICR-MS (+) with a focus at 305 m/z with the molecular assignments and an optical image of* G. prisca.

References

[1] Blokker P, van Bergen P, Pancost R, et al (2001) The chemical structure of *Gloeocapsomorpha prisca* microfossils: implications for their origin. Geochimica et Cosmochimica Acta 65:885–900. https://doi.org/10.1016/S0016-7037(00)00582-2

[2] da Silva TF , Mendonça Filho JG, da Silva MC, et al (2016) *Botryococcus braunii* versus *Gloecapsomorpha prisca* : Chemical composition correlation using laser micropyrolysis-gas chromatography/mass spectrometer (LmPy-GCMSMS). International Journal of Coal Geology 168:71–79. https://doi.org/10.1016/j.coal.2016.08.010

[3] Derenne S, Largeau C, Casadevall E, et al (1990) Characterization of Estonian Kukersite by spectroscopy and pyrolysis: Evidence for abundant alkyl phenolic moieties in an Ordovician, marine, type II/I kerogen. Organic Geochemistry 16:873–888. https://doi.org/10.1016/0146-6380(90)90124-I

[4] Derenne S, Metzger P, Largeau C, et al (1992) Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* as a response to changes in salinity. Organic Geochemistry 19:299–313. https://doi.org/10.1016/0146-6380(92)90001-E

[5] Raevskaya, E., le Herisse, A., & Steemans, P. (2004). Quantitative distribution and evolution of palynomorphs associated with kukersite deposits in the Middle-Upper Ordovician of the East European Platform. International Symposium on Early Palaeozoic Palaeogeography and Palaeoclimate.

[6] Kiipli E, Kiipli T (2013) Nitrogen isotopes in Kukersite and black shale implying Ordovician-Silurian seawater redox conditions. Oil Shale 30:60. https://doi.org/10.3176/oil.2013.1.06

[7] Goesmann F, Brinckerhoff WB, Raulin F, et al (2017) The Mars Organic Molecule Analyzer (MOMA) Instrument: Characterization of Organic Material in Martian Sediments. Astrobiology 17:655–685. https://doi.org/10.1089/ast.2016.1551