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Exceptional Biomarker Preservation and the Role of Sulfur Diagenesis in Kangerlussuaq, Greenland

PROJECT REPORT

Introduction

Determining how animal fossils form and the ways in which their soft tissues are preserved in the geologic record has been a challenge to unravel because decay begins immediately after senescence while diagenetic alteration of organic matter continues over millions of years. Laboratory experiments simulating decay and diagenesis running days to years provide important insights to some of the processes taking place. However, fossilization of organic matter is ultimately the result of compounding and unpredictable parameters (biotic and abiotic: microbial communities, sedimentation rates, sulfurization, authigenesis, reducing vs. oxidizing conditions, etc.) which are challenging to incorporate into comprehensive experiments. Therefore, while taphonomic experiments have significantly advanced our understanding of organic matter preservation, it would be ideal if fossilization could be 'caught in the act' under natural conditions. In order to address this, carbonate concretions containing exceptionally preserved organisms have been utilized as natural instances of decay and diagenesis to provide a window or 'snapshot' into the enigmatic fossilization process. This is motivated by work that has demonstrated concretions preserve signals for the original nucleating organic carbon source, microbial decay communities, the environment, and the continuum of diagenetically altered biomarker byproducts [1-3]. Our objective here is to conduct a comprehensive assessment of fossilization within a modern and understudied (less than 10,000 years ago) Konservat-Lagerstätten location that may permit the first-ever multi-proxy (i.e., lipids, proteins, DNA) analysis of organic matter preservation. Here we investigate the biological and early diagenetic transformation of organic matter within concretions in order to elucidate those processes, biotic or abiotic, which determine subsequent organic carbon preservation and the potential range of products that will arise upon late diagenesis.

What are concretions?

Carbonate concretions are lithified nodules that have a wide diversity of shapes, sizes, and can be characterized by having a sharp discontinuity from their surrounding sediment (Supplementary Figure 1). Concretions are primarily the result of biologically induced mineral precipitation (e.g., authigenesis) encapsulating organic matter. It is generally believed that concretion formation is the result of anaerobic microbial decay which produces a pH and alkalinity gradients that consequently induces mineral precipitation [4]. In some instances, concretion formation may occur fast enough to completely arrest decay at various stages of diagenesis and thus preserve a record of the fossilization process within the natural environment [5].

Early Decay and Diagenesis: Selective pathways for preservation?

During the earliest steps of decay, autolysis by hydrolytic enzymes digest carbohydrates and proteins while labile organic compounds are quickly remineralized by heterotrophs. Furthermore, differences in metabolic potential/preferred redox couples (i.e., oxic vs anoxic conditions) do not necessarily result in increased preservation potential (one over the other). One of the foremost areas of current biomarker research (in organic geochemistry) is in the preservation of organic matter via secondary reactions involving environmental sulfur [6]. For example, in euxinic environments (reducing and H_2S-rich), hydrogen sulfide produced by sulfate reducing bacteria can mediate the hydrogenation of ephemeral unsaturated organic compounds (e.g., carotenoids) and cross-linking reactions that yield decay-resistant macromolecules (e.g., a protokerogen). One hypothesis is that sulfurized compounds (i.e., organically-insoluble macromolecules) are required for stabilizing otherwise labile biomolecules during long-term diagenesis.

Kangerlussuaq, Greenland: A Modern and Understudied Konservat-Lagerstätten

Modern Kangerlussuaq fjord (Danish: Søndre Strønfjord) in West Greenland is a marine environment located between the Davis Strait and at the confluence of meltwater rivers originating from the now land-locked Russell Glacier (Supplementary Figure 2 & 3). The present-day fjord depth varies between a few meters beyond the outwash plain (sandur) by the Kangerlussuaq airport (SFJ) to over 300 meters where the mouth of the fjord meets the Davis Strait. However, during the Last Glacial Maximum (LGM), the Greenland Ice Sheet (GIS) reached further down the Kangerlussuaq valley to create a tide-water system [7]. By ~8,000 years ago, the GIS had continued its retreat inland and the glacier terminus was located near or above SFJ as indicated by the radiocarbon-dated Umîvît moraine system (Supplementary Figure 4) [8]. Sea level has been inferred to have been approximately 40 meters above modern sea-level at this point [8]. This is where exceptionally-preserved capelin concretions containing fully articulated remains and soft-tissue have been found and described since the 1700s [9]. Capelin are small forage fish, which spend most of their life offshore and only enter coastal waters to spawn usually resulting in mass deaths. In recently published work, we conducted a lipid biomarkers analysis and determined that as the GIS retreated, Kangerlussuaq fjord experienced eutrophic and euxinic conditions not unlike neighboring fjords and lakes [5], [10], [11]. It is possible that, because of sulfur-rich conditions, Kangerlussuaq mediated high-preservation potential and should be regarded as a modern Konservat-Lagerstätten (high concentration of exceptionally preserved fossils) location.

Sample Collection at Kangerlussuaq

The best-known fossil outcrops are found south-west of the Kangerlussuaq airport within exposed glaciomarine sediments (67°00′06.8″N 50°46′19.8″W). Concretions can be found along steep margins carved by seasonal outflow channels. A typical ‘fossil hunt’ at Kangerlussuaq involves scrambling alongside these cliffs while scanning the outcrop for recently exhumed concretions. Supplementary Figure 5 details the walk from basecamp to the first outflow channel where the entire south-facing wall was combed before moving onto the north-facing wall and later channels. Supplementary Figure 6 shows a capelin concretion partially exposed on a steep wall, view of an outflow channel from atop, and from the inside. Careful attention was focused on collected unopened capelin concretions that have not undergone weathering and oxidation (Supplementary Figure 7). Supplementary Figure 8 shows opened concretions which display exceptionally preserved soft tissues, hard tissues, and articulation.

Methods

We conduct a lipid biomarker analysis using gas-chromatography mass-spectrometry (GC-MS) of soluble organic matter (freely extractable lipids), insoluble organic matter (i.e., sulfurized-fraction), and pyrolysis (py-GC-MS) of targeted tissue types spatially sampled from each capelin (e.g., vertebra, gill, stomach, muscle, brain, bones) (Supplementary Figure 9). Moreover, we conducted metagenomic and metaproteomic sequencing of ancient DNA and proteins at the University of Copenhagen (results pending). Altogether, results from these experiments allow us to better understand selective preservation processes, diagenetic alteration mechanisms, environmental conditions at time of fossilization and which microbial decay communities are involved in decay and concretion formation.

Results

Environmental biomarker results from Kangerlussuaq are comparable to those of contemporary eutrophic fjords. High concentrations of algal sterols (phytosterols), terrestrial biomarkers (e.g., amyrin), and branched-chain fatty acids confirm massive organic matter input during the deglaciation of Kangerlussuaq valley resulting in intense primary productivity and sedimentary sulfate-reduction [5]. Moreover, cholesterol and cholestanols suggest bacterial reduction of capelin tissues. Interestingly, desulfurized lipids (e.g., isolated and liberated from a crosslinked sulfurized macromolecule) yielded a comparable diversity to freely extractable compounds. Sterols, stanols, steranes, straight-chain fatty acids, branched-chain fatty acids, monounsaturated and polyunsaturated fatty acids, alkane, and terpenoids were detected in both fractions (free and sulfur-bound). This observation is unexpected and suggest that while sulfurization of lipids occurred at Kangerlussuaq, it is not a driving mechanism for preservation. Results from targeted pyrolysis experiments further confirm this. Thiophenic compounds and dimethyl sulfides (from sulfide bridges) characteristic of sulfur-containing macromolecules are either negligible or not detected from pyrolysates (Supplementary Figure 8). Instead, multivariate statistical analysis of tissues reveal preferential preservation between lipid and protein-rich tissues (e.g., fin vs muscle) (Supplementary Figure 9). A tentative explanation is the formation of lipid-protein-sugar condensates from Maillard-like reactions characteristic of melanoidins [12]. These can be identified by the pyrolytic fingerprint of *n*-1-alkene/n-alkane doublets shown in Supplementary Figure 10 which are also observed in protokerogens.

Conclusions

Results from Kangerlussuaq concretions are far more complex and informative than initially expected. Further study is required and is currently ongoing to better understand the spectrum of preservation pathways observed here -- though there appears to be a distinct relationship between lipid/protein abundances and enhanced preservation potential via macromolecular assimilation.

References

[1] I. Melendez, K. Grice, and L. Schwark, “Exceptional preservation of Palaeozoic steroids in a diagenetic continuum,” *Sci. Rep.*, vol. 3, no. 1, Dec. 2013, doi: 10.1038/srep02768.

[2] V. Thiel and M. Hoppert, “Fatty acids and other biomarkers in two Early Jurassic concretions and their immediate host rocks (Lias δ, Buttenheim clay pit, Bavaria, Germany),” *Org. Geochem.*, vol. 120, pp. 42–55, Jun. 2018, doi: 10.1016/j.orggeochem.2018.02.010.

[3] S. K. Lengger, I. M. Melendez, R. E. Summons, and K. Grice, “Mudstones and embedded concretions show differences in lithology-related, but not source-related biomarker distributions,” *Org. Geochem.*, vol. 113, pp. 67–74, Nov. 2017, doi: 10.1016/j.orggeochem.2017.08.003.

[4] R. Raiswell, “The microbiological formation of carbonate concretions in the Upper Lias of NE England,” *Chem. Geol.*, vol. 18, no. 3, pp. 227–244, Oct. 1976, doi: 10.1016/0009-2541(76)90006-1.

[5] A. Mojarro *et al.*, “Comparative soft-tissue preservation in Holocene-age capelin concretions,” *Geobiology*, p. gbi.12480, Nov. 2021, doi: 10.1111/gbi.12480.

[6] P. Adam, P. Schneckenburger, P. Schaeffer, and P. Albrecht, “Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules,” *Geochim. Cosmochim. Acta*, vol. 64, no. 20, pp. 3485–3503, Oct. 2000, doi: 10.1016/S0016-7037(00)00443-9.

[7] R. Dietrich, A. Rülke, and M. Scheinert, “Present-day vertical crustal deformations in West Greenland from repeated GPS observations,” *Geophys. J. Int.*, vol. 163, no. 3, pp. 865–874, Dec. 2005, doi: 10.1111/j.1365-246X.2005.02766.x.

[8] J. E. A. Storms, I. L. de Winter, I. Overeem, G. G. Drijkoningen, and H. Lykke-Andersen, “The Holocene sedimentary history of the Kangerlussuaq Fjord-valley fill, West Greenland,” *Quat. Sci. Rev.*, vol. 35, pp. 29–50, Mar. 2012, doi: 10.1016/j.quascirev.2011.12.014.

[9] O. Bennike, “Quaternary vertebrates from Greenland: A review,” *Quat. Sci. Rev.*, vol. 16, no. 8, pp. 899–909, Jan. 1997, doi: 10.1016/S0277-3791(97)00002-4.

[10] L. Meire *et al.*, “Glacial meltwater and primary production are drivers of strong CO2 uptake in fjord and coastal waters adjacent to the Greenland Ice Sheet,” *Biogeosciences*, vol. 12, no. 8, pp. 2347–2363, Apr. 2015, doi: 10.5194/bg-12-2347-2015.

[11] N. J. Anderson, A. Clarke, R. K. Juhler, S. McGowan, and I. Renberg, “Coring of laminated lake sediments for pigment and mineral magnetic analyses, Søndre Strømfjord, southern West Greenland,” *Geol. Greenl. Surv. Bull.*, vol. 186, pp. 83–89, Jan. 2000, doi: 10.34194/ggub.v186.5220.

[12] S. R. Larter and A. G. Douglas, “Melanoidins—kerogen precursors and geochemical lipid sinks: a study using pyrolysis gas chromatography (PGC),” *Geochim. Cosmochim. Acta*, vol. 44, no. 12, pp. 2087–2095, Dec. 1980, doi: 10.1016/0016-7037(80)90206-9.