Hydrocarbon generation from bacterial biomass in ca. 1320 million years ago

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Abstract Bacteria are the oldest life on the Earth. Some of them, such as cyanobacteria, can produce oxygen and organic matter through photosynthesis. However, the hydrocarbon generation ability of these bacterial biomass is still unclear. Here we reported the molecular evidence from the wrapped bitumen in well-preserved silicified concretions from the Xiamaling Formation, North China. The absence of steranes indicated negligible contribution of eukaryotic algae to the bitumen. Abundant hopanes, 13α(α-alkyl)-tricyclic terpanes and other biomarkers of bacteria prove that bacterial biogenic organic matter can generate hydrocarbons on a large scale. Combined with our previous microfossils and elements studies, these bitumen were considered to be from the Xiamaling shales, for the intrusion of Yanliao large igneous provinces into the organic-rich Xiamaling shales at ca. 1320 million years ago.

1. Introduction

The classical petroleum geology speculated the photosynthetic algae as main sources of sedimentary organic matter and petroleum, while the contribution of bacteria was insignificant or even negligible [1]. As the oldest life on Earth, some of the bacteria, such as cyanobacteria, can produce organic matter through photosynthesis [2]. Although the mean C:P ratio of bacterial biomass was about 78, lower than the Redfield ratio of photosynthetic algae (C:P=106:1), the bacteria could survive and produce organic matter in anoxic even euxinic water bodies, which were common in the pre-Ediacaran ocean [3,4]. For photosynthetic algae, although their molecular fossils as aromatic steroids were date back to ca. 1640 million years ago (Ma) [5], their contribution to the sedimentary organic matter was still lack of sufficient evidence, for the low contents or below the detection limits of conventional GC–MS of steranes in most of the pre-Ediacaran black shales [6-8]. These pre-Ediacaran organic-rich black shales, before the rise of eukaryotic algae at the tail of Cryogenian [6], might be the products of the accumulation of bacterial biogenic organic matter. However, primary oil reservoirs from pre-Ediacaran source rocks were rarely found. Even the source rocks are still within the "oil window", current discoveries are dominated by gas reservoirs [9]. The contribution of bacterial biomass to the hydrocarbon generation are still controversial.

A large amount of bitumen with or without silicified shells were found in the Xiamaling Formation (ca. 1380 Ma), North China. Here, we reported the molecular characteristics of the wrapped bitumen in well-preserved silicified concretions. The alkanes and biomarkers from bitumen extract gave new light into understanding the contribution of prokaryotic bacteria on the organic matter accumulation and hydrocarbon generation in Mesoproterozoic.
2. Geological background

2.1. The Xiamaling Formation

The Xiamaling Formation deposited in the Yanliao Basin, North China, has been precisely constrained to the middle Mesoproterozoic by two zircon U–Pb ages of 1384 ± 1.4 Ma and 1392.2 ± 1.0 Ma from four tuff layers and one K-bentonite layer, at the middle of the Xiahuayuan section [10] (Fig. 1). Before the onset of deposition of the Xiamaling Formation, the Yanliao Basin were considered to experience successive stages of rifting during the Changchengian Period (ca. 1800–1600 Ma) and a passive continental margin stage during the Jixian Period (ca. 1600–1400 Ma) [11]. In our studied areas (Fig. 1C), the Xiamaling Formation was mostly in an unconformity contact with the Changlongshan sandstone (ca. 800 Ma) of Qingbaikouian System, or Jurassic shale or Quaternary loess. The underlying Jixian System are dominated by carbonate rocks and lack of black shale. Thus, the Xiamaling Formation is the only set of black shale in the pre-Jurassic strata.

Previously, we divided the Xiamaling Formation informally into 6 units, for the sedimentary and geochemical fluctuations [12]. Among them, the middle of the Xiamaling Formation (including unit 3, unit 2 and the lower part of unit 1) were main black shales, with a total maximum thickness of ca. 250 m at the Xiahuayuan and Zhaojiashan sections (Fig. 1D) [12,13]. The total organic carbon (TOC) of the Xiamaling black shale ranges from <1% to >10% [10,12,14]. Most of the Xiamaling shales in the Xiahuayuan and Huaihushou sections were never heated to above 90 °C, thus preserving organic biomarkers [15]. However, at the Zhaojiashan and Dacun sections, the Xiamaling shales were intruded by diabases, and the baked shales had much higher maturity than others not. These diabases can be found in almost all of the Yanliao Basin, with a mean age of 1320 Ma [16]. The basin-scale diabase and the contemporaneous Derim–Galiwinku large igneous province (LIPs) of the North Australian Craton might be fragmented parts of the same LIP, named as Yanliao LIPs [16].

![Figure 1](image-url) Figure 1. Locality maps and stratigraphy of the Xiamaling Formation. (A) Location of the North China Craton (NCC). (B) Outcrops of Proterozoic rocks within the NCC. The black rectangle indicates the studied area showed in (C). (C) Regional map and outcrops of Proterozoic rocks in the studied area. (D) Stratigraphic correlation of the Xiamaling Formation at the Xiahuayuan and Huaihushou sections.
2.2. Bitumen in the Xiamaling Formation

Plentiful bitumen with or without silicified shell had been found at the Xiahuyuan section, Hebei Province [17]. A delicate Mesoproterozoic biotic community, including the microfossils and biofilms, were found in some well-preserved bitumen concretions [17]. Great differences of the major and trace elements contents between the silicified shells and surrounding rocks, excluded the possibility of their co-deposition [17]. Much lower contents of total rare earth elements (< 5 μg/g) and positive Eu anomaly of the silicified shells, indicated a possible hydrothermal source [17].

Recently, much more bitumen concretions as an one-meter-thick layer were found above the black shale of the Xiamaling Formation at the Huashugou section (Fig. 1D). Most of the concretions are of ellipsoidal, spindle, or thin pancake structure, with diameter of 1 ~ 5 cm and thickness of 0.5 ~ 2 cm. The discovery of this thick bitumen-rich layer indicated a large-scale hydrocarbon generation.

3. Materials and methods

Six well-preserved bitumen concretions were selected and carefully step-by-step washed by diluted nitric acid, distilled water and then chloroform to remove any possible contamination. Then the concretions were cut by a baked small hacksaw to obtain the wrapped fresh bitumen. Most of the bitumen were filled in the pores of shell and coked, indicating mild or severe thermal alteration. However, there are still some fresh bitumen in spherical or loose features wrapped in the concretions.

Part of the fresh bitumen were selected to measure reflectance (R_0) using MPV-SP microphotometer to determine the maturity. Most of them were crushed to powder and then extract with chloroform for GC-MS analysis. The carbon isotope (δ^{13}C) of each extracted bitumen was analyzed using a Delta V Advantage mass spectrometer (Thermo Finnigan, USA). Each extract was separated into saturated, aromatic, polar and asphaltene these four fractions. The n-alkane and biomarker compositions in each saturated fraction were determined using a Trace GC combined with an Ultra-DSQ II mass spectrometer (Thermo Fisher Scientific). Detail information about the procedures of biomarker and δ^{13}C analysis can be found in our previous work elsewhere [15,18].

4. Results

4.1. Maturity and carbon isotope

The R_0 values of the bitumen varied in a small range of 0.62 to 0.65, with an average of 0.63. The δ^{13}C values of the bitumen also varied in a small range of −32.3‰ to −34.7‰, with an average of −33.6‰. All of these data were in the range of the kerogen values of the Xiamaling shale (Table 1).

|          | S1   | S2   | S3   | S4   | S5   | S6   | Average | Xiamaling Kerogen |
|----------|------|------|------|------|------|------|---------|-------------------|
| R_0 (%)  | 0.62 | 0.64 | 0.65 | 0.62 | 0.63 | 0.64 | 0.63    | 0.6 ~ 0.7         |
| δ^{13}C | −32.3 | −34.4 | −34.5 | −33.1 | −32.8 | −34.7 | −33.6 | −29.3 ~ −35.0     |

4.2. Alkanes and biomarkers

In the bitumen extract, the n-alkanes ranged from C_{12} to C_{31}, with the predominant alkanes of n-C_{17}~n-C_{19} (Fig. 2A). Mono-methyl alkanes were also identified as middle carbon number (C_{15}~C_{22}) methyl isomers (Fig. 2A). Monocyclic-, bicyclic- and tricyclic- alkanes substituted with long-chain n-alkyl were abundant, including the C_{12}~C_{20} alkyl- substituted cyclohexane (m/z 82, Fig. 2B) and bicyclic-alkanes (m/z 137, Fig. 2C), and the latter had two isomers of each carbon.

For the biomarkers, two different series of tricyclic terpanes were identified, including C_{19}~C_{25} 13a(n-alkyl)-tricyclic terpanes (13-NAT Ts, m/z 123, Fig. 3A) and the C_{20}~C_{23} regular tricyclic terpanes (m/z 191, Fig. 3B). Hopanes were abundant in the bitumen extract, including 17α(H)-hopanes (C_{27}~C_{35}, without C_{28}), 17α(H)-di-hopanes (C_{29*} and C_{30*}), 18α(H)-neo-hopanes (Ts and C_{29}Ts) and an early elution component of C_{30+r}bidiahopane (C_{30+r}*) (Fig. 3B). However, the contents of steranes and diasterenes were below the detection limits (m/z 217, Fig. 3C).
Figure 2. GC-MS chromatographs of $n$-alkanes, $i$-alkanes and alkyl- substituted alkanes of the bitumen from the Xiamaling Formation. (A) $m/z$ 85, showed $C_{12}$–$C_{31}$ $n$-alkanes, mono-methyl alkanes and middle carbon number ($C_{15}$–$C_{22}$) methyl isomers; (B) $m/z$ 82, showed long-chain alkyl-substituted $C_{14}$–$C_{28}$ cyclohexane; (C) $m/z$ 137, showed long-chain alkyl-substituted bicyclic alkanes with two isomers for each carbon ranging from $C_{14}$ to $C_{27}$.

Figure 3. GC-MS chromatograms show the mainly bacteria contribution of the bitumen from the Xiamaling Formation. (A) $m/z$ 123, showed $C_{19}$–$C_{25}$ 13α($n$-alkyl)-tricyclic terpanes; (B) $m/z$ 191, showed the distribution of hopanes and diahopanes, the biomarker of bacteria; (C) $m/z$ 217, showed the distribution of steranes and diasteranes, the biomarker of eukaryotes.
5. Discussion

5.1. Biological precursors of the bitumen

Biomarkers are the most common mean to trace and classify the biological precursors [20], however, one critical problem is the source identification, is primary or from post-contamination [21]. Silicified shell and low maturity of the bitumen (Table 1) benefited the primary biomarker preservation. Abundant and long carbon number (C12~C31) n-alkanes indicated weak biodegradation. Strict analysis procedures and the absence of steranes further excluded the possible contamination. So the biomarkers from the fresh bitumen can be used to identify its biological precursors.

Steranes, as the diagenetic and catagenetic products of sterols, which are from eukaryotes, are common used to trace the origin of eukaryotic algae [6]. Their absence or possible extremely low contents in the bitumen extract (Fig. 3C), indicated negligible contribution of eukaryotic algae. Prokaryotic bacteria were speculated as the most possible contributors to the sedimentary organic matter and the generated hydrocarbon, for multiple independent evidence, such as the hopanes (Fig. 3B), long-chain 13-NATTs (Fig. 3A), extended alkyl substitution in monocyclic-, bicyclic-, and tricyclic- alkanes (Fig. 2C). Hopanes are considered to be the most reliable biomarkers of bacteria, and can be detected in almost all of the black shales on the Earth [20]. Especially in the Paleo- and Meso- Proterozoic black shale with extremely low contents of steranes, hopanes were abundant[6]. The abundant extended alkyl- substitution in monocyclic-, bicyclic-, and tricyclic- alkanes in the bitumen extract (Fig. 2C), further indicated important contribution of cyanobacteria [20].

As a special series of compounds, long-chain 13-NATTs were first recognized from the Xiamaling basal bitumen [22]. These compounds were then recognized from the Hongsuizhuang black shale (ca. 1450 Ma) [7,23], while were absence or lower than the detection limits in the Xiamaling black shale and Gaoyuzhuang black shale (ca. 1560 Ma) in the northern of Yanliao Basin [23]. These compounds were also considered as a special series of biomarker [23]. However, long-chain 13-NATTs were also recognized from the Xiamaling shales at the Xiahuayuan and Zhaojiashan sections [8,19]. Furthermore, in our studied area, the Hongsuizhuang Formation was mainly organic-poor green shale without hydrocarbon generation capability. Thus, the recognition of long-chain 13-NATTs indicated that the bitumen might be from the organic-rich Xiamaling black shale. This speculation was also supported by evidence of δ13Corg (Table 1), organic materials maturity (Table 1) and diahopanes. Diapropanes was regarded as diagenetic products of regular hopanoids, and were abundantly detected from the Xiamaling black shales at the Xiahuayuan and Zhaojiashan sections [8,19,24].

5.2. Formation processes of the bitumen concretions

For the bitumen concretions, previous researchers had proposed two possible interpretations. One was that the bitumen was from an vanished mystery hydrocarbon reservoir and deposited with sand after the oxidation and invasion [25]. The other one was that the bitumen was from the hydrocarbon generation of wrapped red algae by silicon rich hydrothermal fluid [19].For the former one, it could not explain the frequent occurrence in almost all of the Xiamaling sediments with a span time of tens of millions of years [17]. For the later one, it was contradict with the absence of steranes.

Based on our previous microfossils and elements evidence [17] and these new finding, we propose an alternative interpretation for the bitumen concretions formation. We speculated that the intrusion of Yanliao LIPs at ca. 1320 Ma might be responsible to the these processes. The Xiamaling shales were baked and generated hydrocarbon, then the hydrocarbons were wrapped by the hydrothermal fluids together with intrusive diabase. Although we did not find the intrusive diabase at the Xiahuayuan and Huaishugou sections, bitumen and diabase were co-existence at the Zhaojiashan and Dacun sections.

6. Conclusions

Molecular analysis of the fresh bitumen in silicified concretions from the Xiamaling Formation prove that prokaryotic bacteria, such as cyanobacteria, were the main biogenetic source of the bitumen. An alternative interpretation was gave that these bitumen might be from the organic-rich Xiamaling shale, for the intrusion of Yanliao LIPs into the Xiamaling shale at ca. 1320 Ma.
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