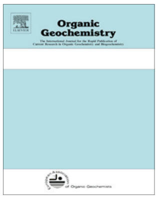
Organic Geochemistry 75 (2014) 36–47

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem



Review

Acyclic isoprenoid biomarkers and evolution of biosynthetic pathways in green microalgae of the genus *Botryococcus* ☆

John K. Volkman *

CSIRO Marine and Atmospheric Research and CSIRO Energy Transformed Flagship, GPO Box 1538, Hobart 7001, Tasmania, Australia
SINOPEC Key Laboratory of Petroleum Accumulation Mechanisms, Wuxi Institute of Petroleum Geology, SINOPEC, 2060 Lihu Road, Wuxi, Jiangsu 213126, China

ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form 12 June 2014
Accepted 12 June 2014
Available online 20 June 2014

Keywords:
Biomarkers
Botryococcenes
Lycopadiene
Botryococcus
Eocene
Biosynthetic pathways
Age-diagnostic
Acyclic isoprenoids

ABSTRACT

Acyclic isoprenoids were among the earliest lipids on Earth and today >30,000 isoprenoid-derived compounds are known, testifying to the remarkable diversification in isoprenoid biosynthetic pathways over time. Many of the isoprenoids have proven to be useful biomarkers in geochemical studies and evidence from sedimentary studies has the potential to provide a timeline for the evolution of different types of isoprenoid biosynthesis. A single green microalgal species termed *Botryococcus braunii* has been recognised as a major contributor of organic matter to sediments as old as the Precambrian. Modern studies of the taxonomy of *B. braunii* using molecular biology techniques have shown that the major clades identified from 18S rRNA gene sequences correspond to races A, B, L and S, which are defined on the basis of their hydrocarbon composition. The biosynthetic pathways by which C_{30}–C_{37} botryococcenes are produced by the B race have now been shown to be due to a duplication of the squalene synthase gene, followed by subsequent changes to the genes, such that one pathway leads to the production of the C_{30} botryococcene and the other to squalene. Both products are then methylated to produce botryococcenes and methylated squalenes having higher carbon numbers. The mode of biosynthesis of lycopadiene in race L is unclear, but may involve coupling of two C_{20} phytyl diphosphates. From an examination of the geological record of botryococcenes and lycopadiene it seems likely that these pathways probably evolved early in the Eocene (ca. 55 Ma) and thus are more recently evolved than the genes for highly branched isoprenoid (HBI) alkenes first produced by diatoms about 92 Ma ago. Botryococcane produced from botryococcenes and a monoaromatic hydrocarbon produced from lycopadiene-related lipids, presumably under anoxic conditions, show promise as age diagnostic biomarkers. In view of these results, it seems likely that the *Botryococcus* species recorded in sediments predating the Eocene lacked the ability to produce botryococcenes or lycopadiene, but nonetheless still contained polymeric non-isoprenoid alkyl chains in the form of an algaenan which on preservation gave rise to a multitude of organic rich rocks.

1. Introduction

Biomarkers, lipids having structures that can be related to a particular biological source (e.g. Eglinton and Calvin, 1967), have proven to be very useful to organic geochemists for assigning the origins of organic matter (OM) in sediments and petroleum (e.g. Seifert and Moldowan, 1981; Volkman, 2006; Bianchi and Canuel, 2011). Detailed inventories of the unique or unusual compounds present in Bacteria, Archaea and Eukarya verify that many compounds can be used as source indicators with a high degree of confidence (e.g. Holba et al., 1998; Brocks and Summons, 2003; Naeher et al., 2014 and references therein). For a biomarker to be preserved in significant amount in sediments at least 3 criteria need to be established: (i) the biosynthetic pathway for the biolipid exists in Nature, (ii) the organisms that carry out this biosynthesis are sufficiently abundant that their organic remains reach the sediment in significant quantity and (iii) the depositional conditions are such that the biolipid is preserved or else are conducive to converting the biolipid to a distinctive diagenetic product. Examples of the importance of anoxia in sediments and/or bottom waters are shown by the presumed conversion of β-carotene (**I**; Appendix) to β-carotane (**II**), squalene (**III**) to squalane (**IV**), C_{34}

☆ Parts of this paper were presented at the AAAPG conference, November 2–4, 2012 in Hangzhou, China and at the International Meeting on Organic Geochemistry, September 15–20, 2013 in Tenerife, Spain.

* Tel.: +61 3 62325281; fax: +61 3 6232 5123.
E-mail address: john.volkman@csiro.au

http://dx.doi.org/10.1016/j.orggeochem.2014.06.005

methylated squalene (**VII**) to C_{34} methylated squalane (**VIII**) and C_{34} botryococcene (**XIV**) to C_{34} botryococcane (X**V**).

The concept of age diagnostic biomarkers, which recognises that some compounds appear in the sediment record at a defined point in time, is now established (e.g. Holba et al., 1998; Moldowan, 2000; Adam et al., 2006; Rampen et al., 2007). It has proven to be a powerful tool in assessing the age of petroleum samples (e.g. Moldowan, 2000; Sinninghe Damsté et al., 2004; Rampen et al., 2007; Barbanti et al., 2011) and for examining the correspondence between the fossil record and biomarker record (e.g. Brocks and Summons, 2003; Taylor et al., 2006).

Advances in molecular biology are increasingly being applied to organic geochemical studies of recent sediments to allow identification of organisms present in the depositional environment (e.g. Boere et al., 2011) and to study the processes involved in cycling of major elements such as C and N in different environmental settings (e.g. Abell et al., 2010). The genetic principles underlying the biosynthesis of some of the biomarkers used by organic geochemists are now being elucidated, although many pathways are still poorly understood or have been shown to be much more complex than first thought. For example, the occurrence of C_{37}–C_{39} alkenones in haptophyte microalgae was first elucidated by organic geochemists many years ago (Volkman et al., 1980), but how they are biosynthesised is still unconfirmed despite plausible pathways being proposed (Rontani et al., 2006).

The role of genetic changes in understanding the evolution of different life forms on Earth is now a central tenet of modern science. In the same way that physical fossils have underpinned our understanding of evolution on earth, the opportunity now exists to use biomarkers to understand the evolution of biosynthetic pathways over geological time (e.g. Pearson et al., 2003; Boucher et al., 2004; Welander et al., 2010; Villanueva et al., 2014) and thus fulfil the promise inherent in the use of the term "chemical fossil" when biomarker science was in its infancy (Eglinton and Calvin, 1967). Phylogenetic studies that show which organisms possess specific pathways are providing a better conceptual basis for understanding why particular biomarkers occur in different organisms and should help establish timelines for the first appearance of a variety of age diagnostic biomarkers. Moreover, this framework should enable us to have greater confidence as to whether or not a particular biomarker is unique to a particular genus or class of organism.

In this paper I review the occurrence of specific isoprenoid biomarkers (botryococcenes and lycopadiene) in different races of the green alga *Botryococcus braunii* and the geological record of biomarkers derived from them. It has been suggested that the *B. braunii* complex of races includes multiple species, although this has to be established. Accordingly, the term "*B. braunii*" will be used here, while recognising that this descriptor may ultimately be shown to include different species. This information, combined with recent advances in our understanding of the biosynthesis of these compounds and the taxonomic diversity of *Botryococcus* revealed by 18S rRNA gene sequences, provides a consistent picture of the evolution of the biosynthetic pathways producing these isoprenoids and suggests that they probably cannot be used as biomarkers for *B. braunii* in sediments and oils older than the Eocene.

2. Results and discussion

2.1. The genus Botryococcus and its biochemical classification

B. braunii is a cosmopolitan, colonial, pyramid-shaped microalga of the order Chlorococcales, class Trebouxiophyceae (not Chlorophyceae as sometimes stated in earlier literature) in the green algal phylum Chlorophyta. It was first described by Kützing in 1849 (cited by Douglas et al., 1969). *Botryococcus* can be found in temperate or tropical oligotrophic freshwater to brackish lakes and occasionally in estuaries and fjords. Each cell is surrounded by a thick fibrous 1,4- and/or 1,3-glucan-containing cell wall (Weiss et al., 2012) and the space between cells is filled with a cross-linked hydrocarbon network, as well as liquid hydrocarbons. Rather unusually, the colonies themselves are enclosed in a retaining wall dominated by arabinose–galactose polysaccharides, which sequester liquid hydrocarbons (Weiss et al., 2012). *B. braunii* is notable for its ability to produce a high amount of hydrocarbons typically around 30–40% of colony dry weight although values as high as 75% (Banerjee et al., 2002) and 86% (Brown et al., 1969) have been recorded. Much of this hydrocarbon oil lies in the extracellular matrix of the colony (Wolf et al., 1985a,b; Banerjee et al., 2002; Weiss et al., 2012).

Algal remains attributed to the genus *Botryococcus* have been identified in sediments ranging from Precambrian to recent (Guy-Ohlson, 1992) and the species is thought to have been a major source material for petroleum generation through the ages (Glikson et al., 1989). It is well known as the presumed source of organic-rich sediments, including torbanite oil shales, also known as boghead coals (Tyson, 1995) that formed from pools in swamp forests in the Carboniferous, and of modern rubbery deposits termed coorongite (Cane, 1977; Wake and Hillen, 1981) found in southern Australia. *Botryococcus* remains have also found in Palaeogene lignites (Traverse, 1955) and organic rich shales (Hofmann et al., 2005). However their identification in older samples is only possible by using transmission electron microscopy (Glikson et al., 1989; Guy-Ohlson, 1992) and, although the remains are remarkably similar to modern *B. braunii*, confirmation of the identification in older sediments from the presence of specific isoprenoid biomarkers has proved elusive (e.g. Sinninghe Damsté et al., 1993; Metzger et al., 2005). I argue that this is simply because the botryococcene and lycopadiene pathways found in specific races of present-day *B. braunii* did not evolve in this species until the Eocene.

B. braunii is found in at least 4 biochemically distinct forms called races A, B or L (Metzger and Largeau, 2005), plus a new race termed S (Kawachi et al., 2012), each of which has a distinctive hydrocarbon composition (Table 1). Race A contains large amounts of long chain C_{25}–C_{31} alkadienes and alkatrienes derived from the C_{18} monounsaturated fatty acid (FA), oleic acid (Templier et al., 1984), as well as complex mixtures of C_{52}–C_{64} *n*-aldehydes (botryals) formed by aldol condensation of fatty aldehydes and epoxides and complex lipids derived from these lipids (Table 1). Race B contains C_{30}–C_{37} unusually branched isoprenoid hydrocarbons referred to as botryococcenes (e.g. **X**–**XIV**; Metzger et al., 1985) as well as squalene (**III**) and methylated derivatives, including mono- di-, tri- and tetramethylsqualene (**V**–**VII**) as minor components (Huang and Poulter, 1989a; Achitouv et al., 2004). Race L contains a C_{40} isoprenoid lycopadiene (**XVI**; Metzger et al., 1990) identified as 2,6R,10R,14,19,23R,27R,31-octamethyldotriaconta-14(E),18(E)-diene (**XVI**) (Metzger and Casadevall, 1987). The L race also contains two epoxides derived from oxidation of each double bond in lycopadiene and a variety of tetraterpenoid ethers termed lycopanerols derived via further reactions (Metzger et al., 2003) and high molecular weight polyaldehydes (Berthéas et al., 1999). The newly defined S race produces C_{18} and C_{20} *n*-alkanes and epoxy alkanes but lacks botryococcenes and lycopadiene (Kawachi et al., 2012) and is thus similar to race A. A summary of the different lipids found in the 4 races of *B. braunii* can be found in Table 1. Note that the cells of each race are very similar in size and morphology.

Botryococcenes show promise as a renewable energy source and their structures are suitable as a hydrocracking feedstock (Metzger et al., 1985; Banerjee et al., 2002). An enormous diversity of botryococcene structures (>60) is now known from algal