PERGAMON

Organic Geochemistry 33 (2002) 1127–1130

Organic Geochemistry

www.elsevier.com/locate/orggeochem

Note

Factors controlling the survival of proteinaceous material in Late Tithonian kerogens (Kashpir Oil Shales, Russia)

A. Riboulleau[a,b,*], T. Mongenot[b], F. Baudin[b], S. Derenne[a], C. Largeau[a]

[a]*LCBOP, CNRS UMR 7573, ENSCP, 11 rue P. & M. Curie, 75231 Paris cedex 05, France*
[b]*CNRS FRE 2400, UPMC, 4 Place Jussieu, 75252 Paris, France*

Received 25 April 2002; accepted 20 June 2002
(returned to author for revision 21 May 2002)

Abstract

Thermochemolysis experiments with tetramethylammonium hydroxide (TMAH), on three Late Jurassic kerogens with contrasting features, confirmed the major role of encapsulation into aliphatic structures for the survival of proteinaceous moieties in kerogens, probably via lipid sulphurisation. It also appeared that (i) some amino acid moieties survived even in the kerogen of a sediment deposited under oxic conditions, although in lower relative abundance compared to the other two kerogens, (ii) the survival of amino acids on geological time scales, within an organic matrix, is probably rather common in kerogens, especially for glycine and alanine, and (iii) thermochemolysis of kerogen sub-units with a lower degree of cross-linking rather than direct thermochemolysis of whole kerogens is recommended to test the presence of amino acid moieties, so as to avoid dilution problems and to increase the accessibility of the TMAH.

1. Introduction

The fate of nitrogenous compounds during sedimentary organic matter formation is a subject of increasing interest (e.g. Stankiewicz and van Bergen, 1998). Nitrogen is mostly present in living organisms as proteins thought to be highly sensitive to degradation and, until recently, the fate of peptides during early diagenesis was considered to be either total remineralisation (dominant) or random recombination with other degraded biomolecules to yield heterocyclic moieties. Recent studies, however, using solid state ^{15}N NMR and thermochemolysis with tetramethylammonium hydroxide (TMAH), showed that nitrogen in refractory organic matter of recent marine or lacustrine sediments ($<10{,}000$ y) mostly corresponds to peptidic material (Knicker and Hatcher, 1997; Zang et al., 2000; Garcette-Lepecq et al., 2001; Knicker et al., 2001). Encapsulation into an aliphatic organic matrix was proposed (Knicker and Hatcher, 1997; Zang et al., 2000) to account for this preservation.

We recently reported the formation of amino acids upon TMAH treatment of the methanol-eluted (polar/high molecular weight) non-acid fraction (MeNA fraction) of the pyrolysate of a 140 Ma-old kerogen (*f top*, Kashpir Oil Shales) indicating that proteinaceous material could be preserved in a kerogen over geological time (Mongenot et al., 2001). In contrast, no amino acids had been previously observed via TMAH treatment of several other kerogens (e.g. del Río et al., 1995; Almendros et al., 1998; González-Vila et al., 2001). These studies used direct TMAH treatment on the kerogen and not on the MeNA fraction. This could have led to dilution of the amino acids by the other themochemolysis/pyrolysis products and/or to lower accessibility of TMAH due to the highly cross-linked structure of the kerogens (the MeNA fraction corresponds to relatively small building blocks of the kerogen with a lower degree of

* Corresponding author at current address: Université Lille 1, PBDS, bât. SN5, 59655 Villeneuve d'Ascq cedex, France. Tel.: +33-3-2043-4110; fax: +33-3-2043-4910.

E-mail address: armelle.riboulleau@univ-lille1.fr (A. Riboulleau).

0146-6380/02/$ - see front matter
PII: S0146-6380(02)00081-5

cross-linking). Also, the survival of amino acids may not be a general feature: it occurred in *f top* kerogen owing to early sulphurisation of carbohydrates and lipids (Riboulleau et al., 2000) and efficient encapsulation of proteinaceous material into the resulting network.

The purpose of the present note is to examine these different points. To this end we compared results from TMAH thermochemolysis of the MeNA fraction of *f top* kerogen with those from (i) the direct treatment on this kerogen and (ii) the TMAH treatment of the MeNA fraction of two other typical kerogens from the same formation exhibiting contrasting features to *f top*.

2. Samples and methods

The three samples were selected for their differences in bulk features and kerogen formation pathways (Table 1): *f top* (aliphatic, S-rich), *aBS base* (poorly aliphatic, S-poor) and *aBS top* (intermediate features). The source organisms and preservation pathways of these kerogens were previously determined through spectroscopic and conventional pyrolytic studies (Riboulleau et al., 2000, 2001).

Kerogen isolation, off-line pyrolyses, isolation of the MeNA fraction from the crude pyrolysates by column chromatography and extraction were performed as previously reported (Riboulleau et al., 2000, 2001). Curie point thermochemolysis–GC–MS of the kerogen was performed at 650 and 358 °C as described by Garcette-Lepecq et al. (2001). Aliquots of the dried fraction of MeNA were dissolved in 300 μl of a 25% solution of TMAH in methanol. This solution was then dried and loaded in small ferromagnetic cylinders with a 650 °C Curie temperature. The GC heating program was 50 °C for 10 min, 50–100 °C at 2 °C/min, 100–300 °C at 4 °C/min. The amino acids were identified on the basis of their fragmentation pattern, by comparison with library and previously published mass spectra (Zang et al., 2000; Knicker et al., 2001), as well as by co-injection of authentic standards.

Table 1
Bulk features of crude rock and kerogen for the selected samples

Sample	Bulk rock[a]		Kerogen[b]				Pathway[c]
	TOC	HI	H/C	O/C	S_{org}/C	N/C	
aBS base	2.3	175	0.96	0.27	0.026	0.021	DR
aBS top	12.0	525	1.35	0.18	0.038	0.023	NS + DR
f top	44.5	699	1.39	0.20	0.068	0.014	NS

[a] Rock Eval data: total organic carbon (%), hydrogen index (mg HC/g TOC).

[b] Elemental analysis of kerogens (atomic ratios).

[c] Main pathway of kerogen formation. DR: classical degradation–recondensation (random recombination of degradation products); NS: natural sulphurisation.

3. Results and discussion

3.1. TMAH treatment of f top kerogen

Extensive cracking of the sulphur bridges occurred at 650 °C and a complex mixture of S-containing pyrolysis compounds was obtained. Thus, the chemolysis products could be best observed at 358 °C. These products are dominated by C_6–C_{18} (maximum at C_{16}) even-carbon-numbered fatty acid methyl esters (FAMEs). This series shows a similar pattern to the fatty acids obtained by conventional pyrolysis of the kerogen (Riboulleau et al., 2000) and by TMAH thermochemolysis of the MeNA fraction (Mongenot et al., 2001). Esters of C_4-C_7 dicarboxylic acids occur in low amounts. Low amounts of *N*,*N*-dimethyl,methyl ester derivatives of glycine and alanine were detected, and also a compound with a base peak at $m/z = 116$ (AA116), probably an α-amino acid (116 corresponds to the *N*,*N*-dimethyl, α-amino acid methyl ester fragment).

Comparison of TMAH thermochemolysis products of *f top* kerogen and of its MeNA fraction showed that (i) only three of the amino acids generated from the latter, those with the highest relative abundances, were detected by direct TMAH treatment of the kerogen and (ii) these amino acids are present in lower amounts compared to the fatty acids: (Gly + Ala + AA116)/C_{16}FAME = 0.24 against 0.63 for the 358 °C thermochemolysis of the MeNA fraction. It therefore appears that (i) dilution problems can be overcome at 358 °C as some amino acids are detected, and (ii) the cross-linked structure of the kerogen only allows poor accessibility of TMAH to amino acid moieties, compared to the MeNA fraction, hence the lower amino acid/FAME ratio. These results support encapsulation of the proteinaceous moieties within an aliphatic matrix; whereas the FAs, linked to this matrix, are more accessible.

3.2. TMAH treatment of the MeNA fraction of the three kerogens

Previous thermochemolysis of the MeNA fraction of *f top* showed that 650 °C is a suitable temperature for TMAH treatment as problems due to extensive dilution are not then encountered and contrary to whole kerogens, no secondary degradation of the amino acids occurs, and higher production of amino acids are observed compared to thermochemolysis at 358 °C (Mongenot et al., 2001). The thermochemolysate of the MeNA fraction of *f top* (Fig. 1a) is dominated by amino acids (as *N*,*N*-dimethyl,methyl esters) with substantial amounts of FAMEs. In addition to glycine, alanine,

aspartic acid and serine, two non-protein amino acids, tentatively identified as α-amino-*n*-butyric acid and homoserine, occur in low amounts. The former, often found in recent sediments (e.g. Keil et al., 1998), originates from partial defunctionalisation of other amino acids such as threonine or cysteine (Bada, 1998). Homoserine, an intermediate in the biosynthesis of protein amino acids, should also be widely distributed; however as far as we know, it has not been described so far in sediments. Two unidentified α-amino acid derivatives ($m/z = 116$ as base peak), including the one observed in the thermochemolysate of *f top* kerogen, also occur in relatively high amounts.

The composition of the thermochemolysate of the MeNA fraction of *aBS top* is similar to that of *f top*. The amino acids are the same and except that serine and homoserine are now absent, their distribution is roughly similar. However, glycine and alanine are comparatively more abundant, and the amino acids occur in lesser amounts compared to the FAMEs (Fig. 1b).

The thermochemolysate of the MeNA fraction of *aBS base* showed substantial differences compared to the other two samples. C_{16} and C_{18} methylethers and C_{10}–C_{14} 1,ω-dimethyl-diethers are found in addition to the FAMEs. Glycine and alanine are the only amino acids and they occur in low proportions (Fig. 1c).

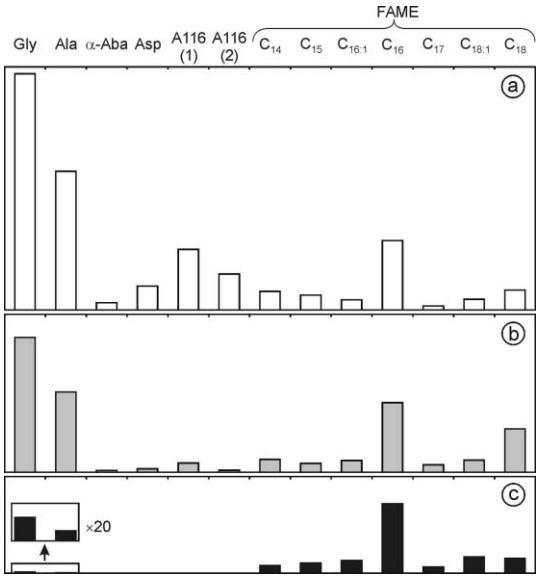

Fig. 1. Distibution of the main products identified in the thermochemolysates at 650 °C of *f top* (a), *aBS top* (b) and *aBS base* (c) MeNA fractions. Gly: glycine; Ala: alanine; α-Aba: α-aminobutyric acid, Asp: aspartic acid; AA116 (1) and (2): unidentified amino acid derivatives. The relative intensities for the three samples are normalised to the C_{16} FAME. Serine and homoserine, present in a very low amount, are not featured. Mass spectral features of the identified amino acids, m/z (intensity). Gly 58 (100), 117 (13); Ala 72 (100), 131 (4); α-Aba 86 (100), 130 (2); Asp 130 (100), 98 (38), 116 (36), 88 (28); AA116 (1) and (2) 116 (100), 114 (11), 128 (5), 173 (3); serine 102 (100), 161 (12), 116 (3); homoserine 116 (100), 175 (14).

The amino acids observed by thermochemolysis of the MeNA fraction of the three kerogens decrease in abundance relative to fatty acids from *f top* to *aBS top* and *aBS base* (Fig. 1). The glycine and alanine contributions relative to the other amino acids show parallel increases, and these are the only amino acids present in *aBS base*. Accordingly, the survival of amino acid moieties on geological time scales within an organic matrix in kerogens is probably a rather common feature, especially for glycine and alanine. Examination of kerogen sub-units, such as MeNA fractions, may be generally required for amino acid detection; indeed such compounds were not detected upon direct THM of the *aBS base* kerogen.

Taking into account the previously-determined preservation pathways and early diagenetic conditions of these three kerogens (Riboulleau et al., 2000, 2001; Table 1), it appears that despite early oxic diagenetic conditions, amino acids were not totally mineralised during the formation of *aBS base* kerogen. However, the bulk of the glycine and alanine moieties retained in this kerogen probably corresponds to secondary products, since glycine and alanine are common diagenetic degradation products of other amino acids (Bada, 1998). These amino acids could be preserved in *aBS base* by (i) encapsulation into the minor portions of the kerogen formed via sulphurisation and/or "oxidative reticulation" as defined by Riboulleau et al. (2001), or (ii) incorporation into the dominant structures formed by random recombination. The inverse correlation observed between amino acid production upon thermochemolysis and the N/C ratio of the *f top* and *aBS base* kerogens (Table 1) makes the latter assumption unlikely and indicates that the bulk of the N in *aBS base* is included in structures that cannot generate amino acids upon thermochemolysis such as highly cross-linked or heterocyclic recombined moieties. A negative correlation is also noted with the O/C ratio, so that oxidative reticulation was probably not important for amino acid preservation. In contrast, the positive correlations between amino acid release and both the H/C and S_{org}/C ratios for the three kerogens indicates that encapsulation in aliphatic sulphurised material was probably the major process for amino acid preservation not only in *f top*, as previously inferred, but also in *aBS top* and *aBS base* kerogens.

Acknowledgements

We thank Dr. H. Knicker and Dr. P. Farrimond for the review.

Associate Editor—**P. Farrimond**

References

Almendros, G., González-Vila, F.J., Martin, F., Sanz, J., Álvarez-Ramis, C., 1998. Appraisal of pyrolytic techniques on different forms of organic matter from a Cretaceous basement in Central Spain. Organic Geochemistry 28, 613–623.

Bada, J.L., 1998. Biogeochemistry of organic nitrogen compounds. In: Stankiewicz, B.A., v. Bergen, P.F. (Eds.), Nitrogen-containing Macromolecules in the Bio- and Geosphere, ACS Symposium Series 707. pp. 64–73.

del Río, J.C., Martín, F., González-Vila, F.J., Verdejo, T., 1995. Chemical structural investigation of asphaltenes and kerogens by pyrolysis-methylation. Organic Geochemistry 23, 1009–1022.

Garcette-Lepecq, A., Derenne, S., Largeau, C., Bouloubassi, I., Saliot, A., 2001. Thermally assisted hydrolysis and methylation of kerogen-like organic matter in a recent sediment off the Danube Delta (northwestern Black Sea). Journal of Analytical and Applied Pyrolysis 61, 147–164.

González-Vila, F.J., Amblès, A., del Río, J.C., Grasset, L., 2001. Characterisation and differenciation of kerogens by pyrolytic and chemical degradation techniques. Journal of Analytical and Applied Pyrolysis 58–59, 315–328.

Keil, R.G., Tsamakis, E., Giddings, J.C., Hedges, J.I., 1998. Biochemical distributions (amino acids, neutral sugars, and lignin phenols) among size-classes of modern marine sediments from the Washington coast. Geochimica et Cosmochimica Acta 62, 1347–1364.

Knicker, H., Hatcher, P., 1997. Survival of protein in an organic-rich sediment: possible protection by encapsulation in organic matter. Naturwissenschaften 84, 231–234.

Knicker, H., del Río, J.C., Hatcher, P.G., Minard, R.D., 2001. Identification of protein remnants in insoluble geopolymers using TMAH thermochemolysis/GC–MS. Organic Geochemistry 32, 397–409.

Mongenot, T., Riboulleau, A., Garcette-Lepecq, A., Derenne, S., Pouet, Y., Baudin, F., Largeau, C., 2001. Occurrence of proteinaceous moieties in S- and O-rich Late Tithonian kerogen (Kashpir oil Shales, Russia). Organic Geochemistry 32, 199–203.

Riboulleau, A., Derenne, S., Sarret, G., Largeau, C., Baudin, F., Connan, J., 2000. Pyrolytic and spectroscopic study of sulphur-rich kerogen from the "Kashpir oil shales" (Upper Jurassic; Russian Platform). Organic Geochemistry 31, 1641–1661.

Riboulleau, A., Derenne, S., Largeau, C., Baudin, F., 2001. Origin of contrasting features and preservation pathways in kerogens from the Kashpir oil shales (Upper Jurassic, Russian Platform). Organic Geochemistry 32, 647–665.

Stankiewicz, B.A., van Bergen, P., 1998. Nitrogen-containing Macromolecules in the Bio- and Geosphere. Oxford University Press, Oxford.

Zang, X., van Heemst, J.D.H., Dria, K.J., Hatcher, P.G., 2000. Encapsulation of protein in humic acid from a histosol as an explanation for the occurrence of organic nitrogen in soil and sediment. Organic Geochemistry 31, 679–695.