FULVIC ACIDS FROM PARTICULATE MATTER OF A WATER-LOGGED PEATLAND

F. MARTÍN and F.J. GONZÁLEZ-VILA

Instituto de Recursos Naturales y Agrobiología, C.S.I.C., 41080 Sevilla (Spain)

Abstract

The presence of aggregates of humic substances (HS) with inorganic adsorbates in a lagoon located in Huelva (south Spain) is reported. The chemical nature of the fulvic acid (FA) fraction, extracted from the natural aggregate by treatment with chelating resins, has been investigated by physical and chemical methods. The results suggest that these FA are similar to FA from other aquatic environments. As revealed by FT-IR and NMR spectroscopies and mild oxidation with potassium persulfate, the FA contain predominantly in their structure polysaccharides and components derived from lignin.

1. Introduction

Humic substances (HS) can affect the surface properties of suspended particulate matter in waters. The adsorption of HS by oxides of Al and Fe in natural waters affects the adsorption of phosphate, silicate and trace metals (Davis, 1984; Tipping, 1984). Knowledge of the adsorption mechanisms is essential to an overall understanding of the geochemistry of the inorganic adsorbates, and to the assessment of the environmental significance of the surface-dependent processes.

However, as far as we know, little attention has been paid to the structure and function of the HS involved in such processes. Organic matter in natural waters can be divided into dissolved and particulate organic carbon, but most of the studies concerned with aquatic HS have been devoted to the former (Aiken, 1985). In water samples from a peat environment there exists probably a dynamic equilibrium between the above-mentioned fractions and an additional colloidal fraction which is difficult to define.

Tipping and coworkers have described the adsorption of aquatic HS onto Fe-oxides in different environments, but they have focused their studies mainly on adsorption, colloid stability, uptake of trace metals and transport processes (e.g., Tipping, 1986).

The purpose of the present paper is to report the presence of a natural HS–metal oxide complex in a lagoon of Huelva (south Spain), the isolation of fulvic acids (FA) from this particulate matter, and their structural characteristics.
2. Materials and methods

The lagoon Las Madres (\(\sim 1 \text{ km} \times 0.3 \text{ km}\)) constitutes a peat deposit exploited commercially for horticultural purposes. The drainage waters are used to irrigate nearby agricultural fields. The peat has an average value of pH of 3.7, and the horizons are fibric in surface and hemic in depth. The botanical composition is a mixture of Cladium mariscus, Phragmites australis and Erica sp.

The water was taken in the dry season when the concentration of particulate matter was highest, collected in 50-l polyethylene containers and stored in a refrigerated room to undergo sedimentation. After the sedimentation of particulates the supernatant water was decanted and the vessels newly filled. The process was repeated until a sufficient amount of particulate matter had been accumulated. After centrifugation the particulates were dialyzed against deionized water to remove water-soluble salts and low-molecular-weight organic compounds and then lyophilized.

To obtain the FA in the H\(^+\) form, the subject of this study, a portion of particulate matter was shaken with a strong-acid chelating resin (Dowex\(^\circledR\) 50 W, H\(^+\) form; Fluka A.G., Buchs, Switzerland) and after 24 hr. the solution, with a pH of 1.5, was separated by filtration, centrifuged, concentrated by vacuum distillation and finally lyophilized. A precipitate of humic acids (HA) was observed. The recovery of FA with a very low ash content (0.5%) was 4.05% of the total organic matter present in the aggregate.

Elemental analysis and functional-group determinations of the samples were carried out according to methods previously described (Martín et al., 1977).

The Fourier transform-infrared (FT-IR) spectrum of the FA was recorded in KBr pellets adding 100 scans at a resolution of 2 cm\(^{-1}\). Electron spin resonance (ESR) measurements were made in quartz tubes and recorded at 297 K employing 100-kHz modulation and a nominal operating frequency of 9.37 GHz. The solution \(^{13}\text{C-NMR}\) (nuclear magnetic resonance) spectrum was obtained in NaOD according to González-Vila et al. (1983).

Persulfate oxidation of the FA at acidic pH was achieved according to Martín et al. (1981). After reaction the digest was extracted with ethyl acetate for 72 hr. in a liquid-liquid extractor. The extract was evaporated to dryness in a rotary evaporator, suspended in methanol and methylated with an excess of diazomethane. The methylated degradation products were analysed by injection into a gas chromatography–mass spectrometry (GC–MS) computer system (Hewlett-Packard\(^\circledR\) model 5992). Experimental conditions for GC–MS studies and methods for identification of components in the mixture of oxidation products were previously reported (González-Vila and Martín, 1985).

3. Results and discussion

Table I shows the chemical composition of the natural aggregates (HS-N) and of the isolated FA fraction (FA-H).

The results found for HS-N are different to those reported by Schwertmann and Fischer (1973) and Tipping et al. (1981) for other naturally occurring water aggregates. The Fe contents of the samples studied by these authors were in the range 20–45%, possibly because the dominant inorganic adsorbates are Fe-oxides, whereas, as shown by IR, ESR and differential thermal analysis (DTA) measurements (not shown here), in the HS-N both Fe-oxides and silicates are present. However, the C content in the aggregates from Las Madres is similar to those reported by the above cited authors. As suggested by Tipping et al. (1981), it is probable that part of the C belongs to organic substrates, such as microorganisms, algae and vegetal debris, not associated with inorganic particles.

The elemental composition of FA-H is similar to FA from different aquatic environments (Steinberg and Muenster, 1985), but is lower
TABLE I

Chemical composition of the natural aggregate (HS-N) and of the fulvic acids extracted from it (FA-H)

<table>
<thead>
<tr>
<th></th>
<th>(%)</th>
<th></th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>10.32</td>
<td>C</td>
<td>43.43</td>
</tr>
<tr>
<td>H</td>
<td>4.22</td>
<td>N</td>
<td>1.26</td>
</tr>
<tr>
<td>ash</td>
<td>78.5</td>
<td>Mn</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>Cu</td>
<td>120</td>
</tr>
<tr>
<td>Zn</td>
<td>540</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. IR spectrum of the FA-H.

in carbon (43.4% compared to 57.5%) than HS isolated from British lakes where the existent aggregates are more closely related to the ones of this study (Tipping, 1984).

The data for functional groups analysis are (expressed in meq g⁻¹) the following: total acidity 12.7, carboxyls 7.6 and phenolic hydroxyls 5.5. These values are characteristic of FA from various origins, including aquatic FA, although in these cases most of the existent data refer to dissolved organic matter.

Fig. 1 shows the FT-IR spectrum of FA-H, exhibiting the characteristic peaks of other FA, i.e. a broad band around 3400 cm⁻¹ (hydrogen bonded OH), a band at 2900 cm⁻¹ (aliphatic C-H stretching), a shoulder between 2880 and 2350 cm⁻¹ (OH of COOH), a sharp band at 1710 cm⁻¹ (C=O of COOH, ketones and quinones), a band at 1625 cm⁻¹ (assigned to aromatic C=C and to H-bonded C=O), a band at 1400 cm⁻¹ (COO and/or aliphatic C-H), a band at 1210 cm⁻¹ (C-O stretch of OH, deformation of COOH) and bands at 1090, 1025 and 975 cm⁻¹ (stretching of polysaccharides).

The ESR spectrum of the FA-H shown in Fig. 2 is similar to those reported by Senesi et al. (1977) for naturally occurring Fe-FA complexes and podsol FA with low ash content. It consists of two main signals, one asymmetrical resonance at \( g = 4.16 \) with an average line-width of 125 G, and the other an isotropic band with a line-width of 5 G at \( g \)-values ranging from 1.99 to 2.002. This resonance and its characteristics (line-width, 5 G; free radical concentration, \( 1.4 \times 10^{17} \) spins/g) is similar to those commonly observed for humic materials, indicating the presence of free radical species of semi-quinonic nature, highly conjugated with an extended aromatic network. To this resonance near \( g = 2 \) spin interactions of neighbouring Fe(III) ions in the material probably also contribute, although it is not certain whether this Fe(III) is bound at individual organic complexing sites or is present in oxides or clays.

The resonance at \( g = 4.16 \) has been attributed by Senesi et al. (1977) and Schnitzer and Ghosh (1982) to strongly bound Fe(III) ions and protected either by tetrahedral or octahedral coordination via COOH and phenolic OH groups, although McBride (1978) was not able to produce the \( g = 4.1 \) signal by addition of Fe(III) to FA.
These assignments are in accordance with the chemical analysis (Table I), which shows that Fe is the major paramagnetic element among the ash metals.

Fig. 3 shows the $^{13}$C-NMR spectrum of the FA-H. The spectrum is poorly resolved and presents only broad absorption bands rather than peaks, as may be expected for complex molecular systems, although its resolution is similar to those solution $^{13}$C spectra of FA from other environments, including seawater (Stuermer and Payne, 1976) and fresh water (Wilson et al., 1981).

The $^{13}$C-NMR spectrum of the FA-H consists of a number of aliphatic resonances in the 20–50-ppm region devoid of well-defined signals for terminal methyl groups (15–20 ppm) or $-\text{CH}_2$ in long alkyl chains ($\sim 30$ ppm), which have been typically found in other FA. The maxima of intensity in this region appear at 36 and 40 ppm, which are typical chemical shifts for aliphatic carbon atoms neighbouring to C–OH groups (Breitmaier and Voelter, 1974). This possibility is supported by the presence of characteristic peaks corresponding to glucosidic structures in the region between 50 and 100 ppm. Thus, the peak at 62 ppm represents the resonance of the C-6 carbon atom in sugars of amylose or celllobiose types; the peaks at 70 ppm arise from carbohydrate ring-carbon resonances and the discernible signal at $\sim 105$ ppm corresponds to the anomeric carbons of polysaccharides. The simultaneous occurrence of these resonance bands suggests a high proportion of carbohydrates. This result agrees with the finding of Painter (1983) that aquatic humus from peat is primarily carbohydrate in origin and also with the view that the melanoidin reaction, which involves carbohydrates, play a significant role in the process of the formation of the core of HS in nature (Steinberg and Muenster, 1985; Ikan et al., 1986). An estimate of the amount of polysaccharide carbon atoms obtained by integration of the spectrum accounts for $\sim 40\%$ of the total carbons. This value is in disagreement with the analytical determination of total sugars by the colorimetric anthrone test, 2%. The high discrepancy between these values is indicative that both methods are not particularly suitable for an exact determination of carbohydrates in such a complex matrix (Stabel, 1977; González-Vila et al., 1983).

In comparison with the $^{13}$C-NMR spectra of other FA, which show predominantly aliphatic signals and present virtually no intensity in the aromatic regions, the FA-H show broad ill-defined structural bands with a maximum at 125–129 ppm, typical of unsubstituted aromatic carbons, although the contribution of heterocyclic structures detected among products of the melanoidin pathway (Ikan et al., 1986) should not be excluded. The low intensity of the resonance contrasts, however, with the prominent aromatic bands of the lake FA.
TABLE II

<table>
<thead>
<tr>
<th>Persulfate oxidation products of FA-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic acid</td>
</tr>
<tr>
<td>Glutaric acid</td>
</tr>
<tr>
<td>1,2-Benzenedicarboxylic acid</td>
</tr>
<tr>
<td>1,3-Benzenedicarboxylic acid</td>
</tr>
<tr>
<td>n-C_{18} fatty acid</td>
</tr>
<tr>
<td>3-Hydroxy-1,2-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>2-Hydroxy-1,5-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>1,8,5-Benzenedicarboxylic acid</td>
</tr>
<tr>
<td>n-C_{18} fatty acid</td>
</tr>
<tr>
<td>3,4-Dihydroxy-1,2-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>4,5-Dihydroxy-1,2-benzenedicarboxylic acid</td>
</tr>
<tr>
<td>5-Hydroxy-1,3,4-benzenetricarboxylic acid</td>
</tr>
<tr>
<td>4-Hydroxy-1,2,3-benzenetricarboxylic acid</td>
</tr>
<tr>
<td>1,2,3,4-Benzenetetracarboxylic acid</td>
</tr>
<tr>
<td>1,2,4,5-Benzenetetracarboxylic acid</td>
</tr>
<tr>
<td>5-Hydroxy-1,2,3,4-benzenetetracarboxylic acid</td>
</tr>
<tr>
<td>2-Hydroxy-1,3,4,5-benzenetetracarboxylic acid</td>
</tr>
<tr>
<td>Benzenepentacarboxylic acid</td>
</tr>
</tbody>
</table>

reported by Wilson et al. (1981). Again the melanoidin hypothesis could also clarify the discrepancies in aromaticity values of freshwater HS in terms of different quantitative contributions of carbohydrates to the formation of the HS in different environments.

The resonance at \( \sim 176.8 \text{ ppm} \) is due to carboxyl carbon, including probably those from uronic acids (González-Vila et al., 1983).

Thurman and Malcolm (1983) emphasize the importance of degradation by oxidative procedures in the study of the structure of aquatic HS, although much of the information is dependent on the oxidizing agent and method used.

Instead of alkaline permanganate (a drastic degradation agent), milder oxidative conditions have been tried such as the degradation with potassium persulfate (Martín and González-Vila, 1983). Table II shows the oxidation products obtained from FA-H after persulfate treatment.

Persulfate oxidation produces very few compounds and methylation prior to oxidation failed to increase the variety and yield of the oxidation products. The identified compounds belong to the series normally found (Schnitzer, 1977; Stevenson, 1982; Martín and González-Vila, 1984) by degradation of HS from quite different origins, i.e. monocarboxylic acids, dicarboxylic acids, benzenecarboxylic acids and phenolic acids. It is noticeable that most of the oxidation products are aromatics and similar to those released by persulfate oxidation of soil FA (Martín and González-Vila, 1983). The presence of phenolic acids, typical lignin degradation products, as well as their predominance over benzenecarboxylic acids denotes a lignin origin, although according to Reuter et al. (1983) this ratio may be only indicative of the reaction conditions, and therefore should be considered with caution. The lignin origin is in agreement with the assumed predominant terrestrial origin for HS in freshwater ecosystems (Gjessing, 1976). However, in addition to lignin, plant polyphenols could be precursors for a number of compounds listed in Table II, and some of them may be synthesized by microorganisms (Neyroud and Schnitzer, 1974). Therefore, it is reasonable to assume that different sources, including autochthonous mechanisms, could contribute to the formation of the HS under study.

In conclusion, FA isolated from the natural aggregates present in Las Madres water-logged peatland have similar structural characteristics to FA extracted from some soils and other aquatic environments, as revealed by physical methods, elemental and functional-group analyses and mild degradative oxidation. The isolated FA seems to contain predominantly in their structure polysaccharides and components derived from lignin.

Acknowledgements

We thank Dr. E. Tipping of Freshwater Biological Association Cumbria (Great Britain) for his critical review of this manuscript and Mr. Aberle of Regensburg University (F.R.G.) for the ESR measurements.
References


