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Fulvic Acids as Transition State of Organic Matter: Indications from High Resolution Mass Spectrometry

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Fulvic acids are one of the largest classes of dissolved organic matter, but they are poorly defined and of unclear origin. Three fulvic acid isolates of different origin were analyzed by size-exclusion chromatography coupled to electrospray ionization-Fourier-transform ion cyclotron resonance (FTICR)—mass spectrometry, and molecular formulas for 700—1900 species in these isolates were derived. All three isolates show the same pattern in the elemental composition of their molecules and a large congruence in the molecular sets. It is proposed that the elemental and structural regularity of fulvic acid molecules does not indicate one common precursor material and formation process, but that this regularity is due to both the strong reworking of source materials in the environment and the valency of the three elements (C, H, O) from which most fulvic acid molecules are formed. Potential molecular formulas of fulvic acids were predicted for a mass range of 60 amu based on a few presumptions. A good agreement was found between the predicted and the detected molecular formulas, and it is concluded that (poly-)carboxylic acids with very limited number of hydroxy groups are the major compound class in fulvic acid isolates. It appears that fulvic acids are metastable molecules that characterize a state of transition of diverse precursor compounds during their oxidation.

Introduction

Fulvic acids are, together with humic acids, the major components of dissolved organic material in natural systems, and they are omnipresent in the soil environment, in groundwater, in freshwater, and in marine systems. With a portion of up to 30—50% of the total dissolved organic matter in natural waters (*1*) fulvic acids contribute substantially to the transport of organic carbon from biosphere to the subsurface, and finally, from land to sea of about 0.4 Gt/yr as well as to the marine carbon budget of about 600 Gt.

Despite of these very large amounts of fulvic acids that are being formed and transported on a global scale, and despite of decades of research, fulvic acid molecules have remained nebulous. This is primarily due to the fact that neither a clear chemical definition of fulvic acids has been derived yet nor that the sources are known from which they are formed. These two aspects are, obviously, closely linked to each other as it is difficult to answer the question on sources as long as the chemical identity of fulvic acids is unknown.

To date, fulvic acids are only operationally defined by their sorption properties toward a certain polymeric sorbent (*2*). However, considerable progress concerning the chemical identity of fulvic acids has been made possible with the invention of electrospray-ionization mass spectrometry (ESI—MS) that made intact fulvic acid molecules amenable to mass spectrometric analysis. It was rapidly recognized that fulvic acid mass spectra exhibit a striking pattern of repeating signal increase and decrease with a periodicity of 14 amu (*3*, *4*, *5*). This similar pattern was, however, also found in crude oils (*6*). A major step forward was provided by high-resolution mass spectrometry that allowed us to analyze individual molecular species out of the complex mixtures and to determine their elemental composition (*4*, *7*—*9*). Together with data gathered by tandem mass spectrometry (*3*, *8*, *10*, *11*) these investigations showed that fulvic acids exhibit elemental and structural regularities that make them a unique and homogeneous class of organic molecules, which is not linked to any other class of organic compounds: (a) their generic structure consists of a more or less saturated carbon skeleton with carboxylate groups as the most prominent functional groups (*8*, *12*). The number of hydroxy groups is limited, whereas ether and ester bonds do not seem to be prominent. (b) Their molecular formulas could be arranged in series of elemental homologues of hydrogenation, methylation, and oxidation (*8*). Beyond these regularities, there is increasing evidence that the sets of molecules in fulvic acid isolates of different origin show a remarkable degree of congruence in terms of their elemental composition and structure (*9*, *13*).

With increasing use of ESI—MS for the analysis of natural organic matter (NOM) some limitations are also seen more clearly:

(a) Electrospray ionization (ESI) is selective as not all classes of organic compounds are detectable by this ionization and ionic compounds are preferred over non-ionic ones. This may not be too problematic for this study, as not NOM, but fulvic *acids* are analyzed which are known to be rich in carboxylic acid groups, and thus, ideal molecules for detection by ESI—MS in the negative ion mode. Anyhow the sole operational definition of fulvic acids makes it impossible to compare the molecules made visible by ESI—MS with what should be seen, because the latter is still unknown.

(b) A number of labile organic molecules in such isolates may be subjected to fragmentation in the ESI source, and thus, a part of the ions detected by mass spectrometry may orginate from fragments rather than from intact molecules. When analyzing molecules of unknown elemental composition and structure, such fragmentations are hardly recognizable, but especially ester and ether bonds could be prone to such in-source fragmentations. With size-exclusion chromatography coupled to ESI—MS it was shown that fulvic acids of lower molecular mass are detected more sensitively than those of higher mass and that low molecular weight ions occur in the high molecular weight fraction (*14*).

Anyhow, recent mass spectrometric findings have fueled the discussion whether fulvic acids are formed from specific precursor material via defined pathways (*10*, *11*). Indeed, the improved knowledge on the elemental composition and potential structures of individual fulvic acid molecules now provides new access to the second fundamental question mentioned above, that about fulvic acid sources. However,

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the thousands of elemental formulas that are accessible by modern ESI−high resolution−MS may confuse, rather than help, in this process.

It was a widely accepted paradigm of humic and fulvic acid research that these fractions represent highly refractory organic matter that may contribute to organic carbon storage and transport but does not participate in organic matter transformation. Contrary to that, there is increasing experimental evidence that this material is not inert. Humic acids have been shown to interact with the redox cycle of iron in natural systems (*15*) as well as to act as reductant of organic compounds (*16*) and they experience significant structural alteration upon microbial activity (*17*). As far as highly water soluble fulvic acids are concerned it seems obvious that the steady release of such material with percolating water from surface soils requires both its continuous formation and its continuous degradation.

We coupled, for the first time, size-exclusion chromatography (SEC) with ultrahigh resolution FTICR mass spectrometry to analyze the molecular composition of fulvic acid isolates. The SEC preceding the MS analysis is advantageous as it provides a coarse fractionation according to the molecular size of the analytes (*5*) while the FTICR−MS provides sufficient mass resolution to resolve individual molecular species from the highly complex fulvic acid mixtures. It also provides the mass accuracy to calculate molecular formulas for these molecular species over a wide mass range (*7*, *9*, *10*).

In this study special emphasis has been given to the question of how the large sets of molecular formulas of fulvic acids in an isolate could be arranged and what kind of system one could detect in these data. Additionally, fulvic acid isolates of different origin were analyzed to verify whether the same system can be found in all of them and which differences can be seen in the molecular composition of these isolates. A broadened knowledge on what fulvic acid molecules share with each other and what distinguishes them from other classes of natural organic matter can be a basis for a future chemical definition of this class of compounds or its major components. Such a chemical definition appears to be a prerequisite to end the speculation on precursors of fulvic acids and their formation processes.

Materials and Methods

Reference Materials. The following fulvic acid reference materials were purchased from the International Humic Substance Society (IHSS): Suwannee River fulvic acids (river isolate), Waskish Peat fulvic acid (peat elutriate), and Nordic aquatic fulvic acid (isolated from a tarn with strong influence from a neighboring fen).

Size Exclusion Chromatography. SEC was performed using a Merck-Hitachi HPLC-system with a PL Aquagel (Shropshire, UK) column from Polymer Laboratories with a molecular weight range of 100−30 000 Da, 250 × 4.6 mm i.d. and a particles size of 8 μm, using a flow of 0.3 mL/min of 60/40 (v/v) water/methanol + 5mM NH_4HCO_3. Details were given elsewhere (*5*). A 40 μL volume of a 2 g/L fulvic acid solution was injected. The column effluent was split by a T-piece with a flow of about 10 μL/min directed into the electrospray-interface of the mass spectrometer. This SEC fractionation separated fulvic acid isolates in up to three fractions (e.g., ref *5*), of which only the intermediate fraction of moderate molecular weight (MMW) that eluted about 0.5 min before the LMW fraction is considered here.

Mass Spectrometry. The mass spectrometer used was a Finnigan LTQ FTICR−MS (Thermo Electron Co., Bremen, Germany) linear iontrap FTICR hybrid mass spectrometer with a 6 T superconducting magnet. Instrumental settings were as follows: spray voltage, −4.3 kV; shealth gas, 15 arbitrary units; transfer capillary temperature, 230 °C; transfer capillary voltage, −47 V; tube lens voltage, −140 V; number of injected ions (ICR cell), $0.5 - 1 \times 10^6$. In all experiments negative ion were aquired with a mass resolution of 100 000 (at m/z 400). The instrument was externally calibrated each day by the standard procedure using ascorbic acid and Ultramark 1620 as calibrants. Mass spectra were recorded from m/z 200 to 700 with a scan rate of 1 s. MS data were analyzed with Xcalibur version 1.4 SR1. Molecular formulas were calculated from the exact masses assuming that the number of O > 2, of H > 4, of C > 4 and of double bond equivalents (DBE) $\geq = 1$ for odd electron anions. Only those formulas were accepted that showed $\leq = 1$ mDa deviation from the measured mass.

A number of 7−8 mass spectra recorded in a time window of 0.25−0.3 min of the MMW fraction of each of the three isolates were accumulated to one spectrum. From this spectra the weakest 15% of the molecular signals were excluded, to avoid that noise influences the statistical evaluation and the remaining molecular formulas were compared to those found in the river isolate. For the tarn, 687 out of its 800 molecular formulas were also found in the river isolate, and for the peat elutriate, 597 out of 741 formulas agreed with the river isolate.

Results

A section of a mass spectrum of a fulvic acid isolate is shown in Figure 1a. It illustrates the wavy intensity distribution with a period of 14 amu that is usually detected in fulvic acids. Each nominal mass is occupied by several isobaric ions that differ in their exact mass and that can be resolved by high magnetic field FTICR mass spectrometry (Figure 1b). As fulvic acid ions detected by ESI−MS span over a mass range of several hundreds up to one thousand amu, and as each (second) nominal mass is occupied by a number of isobaric ions, several thousands of molecular species can be determined by FTICR−MS in fulvic acid or NOM isolates. The accuracy of FTICR−MS can be sufficient to calculate unambiguous molecular formulas for a large number of these detected ions (*4*, *7*, *10*, *18*).

In this investigation, in which SEC was coupled to FTICR−MS, molecular formulas of 1900 molecules of even molecular mass in the mass range of m/z 250−670 were determined in the three SEC fractions of one isolate of a river (Suwannee River, IHSS standard). These molecules consist solely of C, H, and O, while nitrogenous molecules were not considered in this study. More than 4000 formulas have previously been published from the same standard fulvic acid material in the range m/z 320−1100 (*10*). 74% of the molecular formulas derived in this study agree to the previous data. Considering that FTICR−MS analyses in these two studies differ in the mode of injection (SEC vs flow injection) and the ionization mode (negative vs positive) as well as in the instrument used and its operational parameters, this is a reasonable agreement.

A so-called van Krevelen diagram can be used to display compositional characteristics of fulvic acids of an isolate in terms of the H/C- and O/C-ratios of its molecules (Figure 1c) (*9*, *19*). But as it normalizes to the carbon number, a van Krevelen diagram sacrifices a lot of information and cannot illustrate changes with regard to the molecular mass of the molecules.

Regularity in Elemental Composition. As an alternative we propose to visualize the molecular composition of a fulvic acid isolate by plotting the number of carbon atoms of each molecule against its molecular mass (Figure 1d). All molecules with a constant sum of their C and O atoms per molecule arrange themselves in distinct island-like areas and the sum C + O can be used to denote each island (Figure 1d, top axis). When the relative signal intensity of each molecular species is added as the third dimension, these data can be display in a contour plot (Figure 1e). Similar patterns are visible,

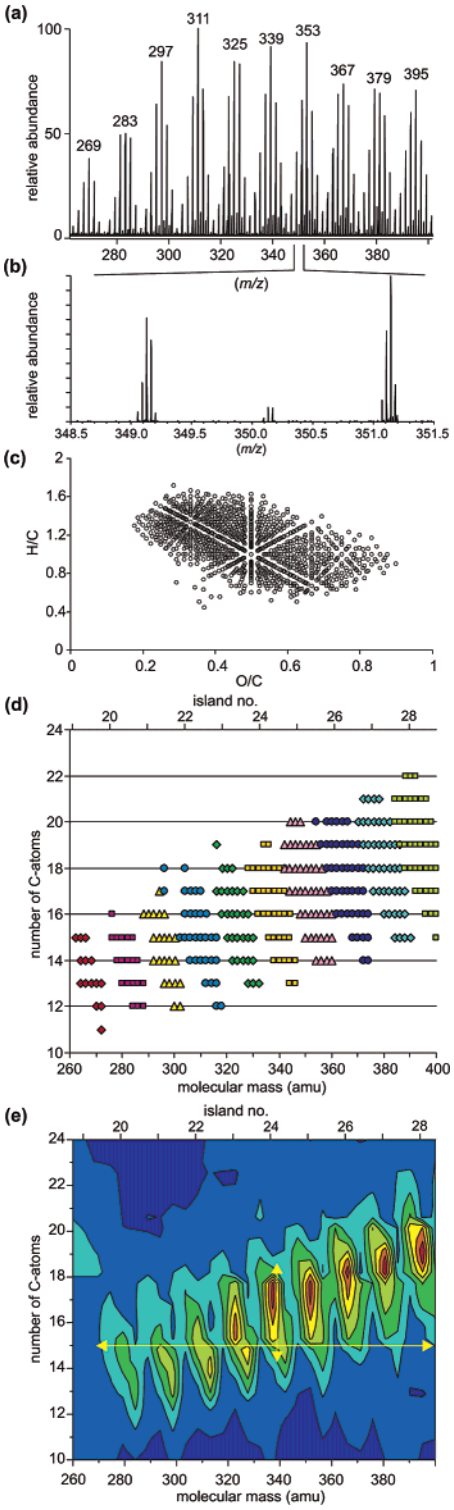

FIGURE 1. (a) FTICR−MS spectrum (*m/z* 260−400) of the river water fulvic acid isolate (second SEC fraction). (b) Extension of spectrum (a) for the *m/z* range 348.5 to 351.5 (c) Van Krevelen-diagram showing the H/C− versus O/C-ratios of 1900 molecular formulas calculated from FTICR−MS data of all three SEC fractions of a river fulvic acid isolate. Not all 1900 dots are visible due to overlay of elemental ratios. (d) Plot of the C-number versus molecular mass for 329 fulvic acid molecules in the mass range 260−400 amu (second SEC fraction). (e) Contour plot of the molecules of (d) with the relative signal intensity as the third dimension. The two yellow lines mark the series of fulvic acids shown in Figure 2 (horizontal line Figure 2a; vertical line Figure 2b). Please note that fulvic acids do not form a continuum in this diagram, but occupy only defined points (see d).

also, in O-number vs mass or H-number vs mass diagrams (not shown). Considering the putative variation in ionization efficacy for molecules of different elemental composition and structure these relative signal intensities do not correspond to relative concentrations. They can, however, be utilized to compare the molecular composition of different samples.

The maxima of these islands have an average distance of 14 amu (corresponding to either $+CH_2$ (14.016 amu) or +O−2H (13.979 amu)). This regularity clearly reflects the 14 amu-periodicity in the fulvic acid mass spectrum (Figure 1a) as each island corresponds to one such period. From one island to the next the sum of carbons and oxygens of its molecules increases by one. In the mass range m/z 211−495 we detected 19 of these islands but this pattern continues toward higher masses.

Within each island the elemental composition changes systematically from one molecule to the next, with clear consequences also for the structure of these molecules:

Horizontally (Figure 1d and e, left to right) within one island the saturation increases stepwise (+2H = +2.0157 amu). From one island to the next, an additional oxygen atom is introduced (+15.9949 amu). The systematic change in elemental composition in horizontal direction is exemplified for the 79 C_{15} fulvic acids of the river isolate in Figure 2a. These C_{15} fulvic acid molecules span over 12 islands, from island #17 ($C_{15}H_{14}O_2$ ($[M\text{-}H]^-$: m/z 225.0923) to island #28 ($C_{15}H_{16}O_{13}$ ($[M\text{-}H]^-$: m/z 403.0507) and exhibit an O/C-ratio between 0.133 and 0.866 (Figure 2a).

Vertically (Figure 1d and e, bottom to top) molecules differ by the exchange of one oxygen by (C + 4H), leaving the sum of C+O unchanged. This replacement leads to the previously recognized mass difference of 0.0364 amu between isobaric ions with the same integer mass (Figure 1b) (*4*, *8*), as illustrated in more detail for fulvic acid molecules with 340 amu in Figure 2b. We detected five of these isobaric molecules in the river isolate (from 340.04305 ($C_{14}H_{12}O_{10}$) to 340.18859 amu ($C_{18}H_{28}O_6$).

Prediction of Possible Elemental Formulas. More use can be made of elemental formulas if they are combined with structural information on the molecules that has been obtained by tandem mass spectrometry. Previous investigations have consistently shown that the fulvic acid molecules detected by ESI−MS are dominated by carboxylate moieties with a limited number of hydroxy groups (*3*, *8*, *12*). With this knowledge, and considering the valency of the three elements C, H, and O, one can predict all chemically allowable elemental formulas of fulvic acids in a certain mass range. This can well be done utilizing the graphical representation in a C− vs mass diagram.

Within one "island" with a constant sum of C + O, two series have to be considered. (i) A *horizontal* series of molecules can range from the completely unsaturated (lowest possible mass) to a fully saturated (highest possible mass) carboxylic acid. (ii) The range in elemental composition in *vertical direction* (exchange of O against (C + 4H)) can also be predicted for each nominal mass. The highest carbon number of such a group of isobaric ions is reached when only two oxygen atoms remain in the molecule to form a monocarboxylic acid (see structure proposal for $C_{22}H_{44}O_2$ at the top end in Figure 2b). The lowest possible carbon number is reached when either the carbon skeleton becomes too small to accommodate more carboxylate moieties or when the number of hydrogens becomes scarce (see structure proposal for $C_{13}H_8O_{11}$ at the bottom end in Figure 2b).

The regions of possible elemental formulas of fulvic acids form triangles with a broad top and a narrow bottom (Figure 3), owing to changes in the structure of the molecules brought about by the exchange of O against (C + 4H). Molecules situated in the upper section of one island are characterized

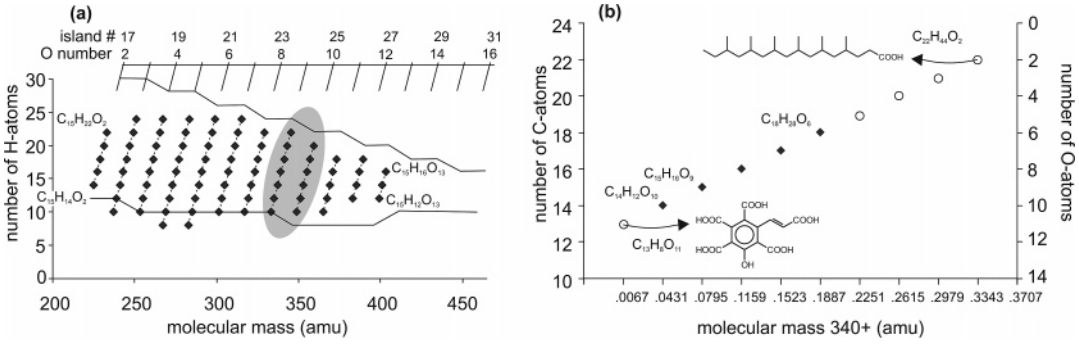

FIGURE 2. (a) Changes in the molecular composition (H vs molecular mass) of all C_{15} fulvic acids determined in the river isolate (black dots). The dotted lines connect C_{15} fulvic acids that belong to one island (same oxygen content). Molecules in the gray area are shown in Figure 3. The solid lines mark the boarders of the predicted elemental compositions of C_{15} fulvic acids in terms of their hydrogen content (top, bottom). (b) Changes in the molecular composition (C vs molecular mass) of all fulvic acids with 340 amu determined in the river isolate (black dots). The open circles mark predicted possible molecules that were not found in the river isolate. Structures proposals are given for the end members. Note that the sum of carbons (left axis) and oxygens (right axis) for all molecules sums up to 24 (island no. 24).

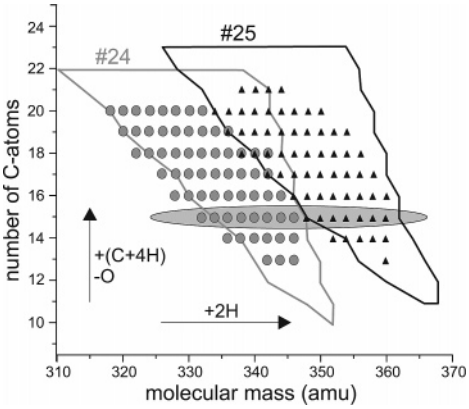

FIGURE 3. Area of predicted fulvic acid molecules in a C− vs molecular mass diagram for the mass range *m/z* 310−370 (marked by the lines) and fulvic acid molecules detected by SEC−FTICR−MS in the river isolate (dots (island no. 24) and triangles (island no. 25)). Molecules in the gray area also shown in Figure 2a.

by a relatively high number of carbon atoms (maximum: island number −2) and, thus, have an extended carbon skeleton. Those require a large number of hydrogenation steps form completely unsaturated (left end) to fully saturated (right end) resulting in a wide extension in *x*-direction (Figure 3). With decreasing C-content and increasing O-content per molecule, the carbon skeleton becomes continuously smaller, while an increasing number of primary carbons is required to accommodate the carboxylate groups (see molecular structure at bottom of Figure 2b). Finally there is only one possible number of hydrogens that allows us to draw a structure according to the structural requirements of the carboxylic acids (bottom angle of each triangle; Figure 3).

Indeed, most of the 125 identified molecular formulas of the river fulvic acid in the mass range 316−360 amu that belong to the island nos. 24 and 25 lie inside the predicted range (Figure 3). The few molecules outside of the respective island exhibit a lower hydrogen content than expected and may be artifacts generated in the mass spectrometric interface by dehydration. Such dehydration reactions that lead to anhydrides have previously been observed for polycarboxylated model compounds (*12*). The reason for the lack of detected molecules on the right side of the two islands (Figure 3) is discussed below.

Comparison of Different Isolates. It was recently shown that the pattern of molecules detected by ESI−MS in fulvic acid isolates of different origin, from a river, a tarn, and a peat elutriate largely agreed (*13*). But due to the lower mass resolution of the mass spectrometer used in that investigation, the comparison was limited to a narrow range of about 75 amu in a low mass range (<300 amu), resulting in a comparatively small set of about 100 molecules. Using FTICR−MS this comparison is now extended to a much wider mass range (250−670 amu), covering about 800 anions in the MMW fraction. Even for this much larger range of molecules a remarkable agreement could be found. A total number of 86% of the molecular formulas detected in the tarn isolate and 80% of the molecular formulas in the peat elutriate were identical to molecular formulas found in the river isolate. More importantly, all formulas detected in the other two isolates also followed the elemental pattern described above. Thus, the molecular composition of fulvic acid isolates of different origin is highly congruent. This confirms recent findings by infusion-FTICR−MS that marine and terrigenous DOM isolates show a large agreement in their molecular composition (*9*).

Discussion

As illustrated in Figure 1, a highly systematic pattern exists in mass spectra and in the molecular composition of fulvic acids seen by ESI−MS. The same has previously been shown for the structure of fulvic acid molecules on the basis of MS/MS spectra that proved carboxylate groups to prevail, accompanied by a few hydroxy groups (*8*, *12*). One could assume that this highly systematic pattern of elemental composition of fulvic acid molecules that was made visible by the application of high resolution-MS in this and previous studies was preserved from the biopolymer precursor material. To preserve such a pattern, only a limited number of specific transformation reactions should act on one dominant class of biopolymers as precursor material of fulvic acids. For several reasons this seems unlikely, at least for the fulvic acid molecules seen by ESI−MS:

(i) The elemental composition of fulvic acid molecules in one isolate is quite broad and covers the characteristic average elemental ratios of different compound classes (*19*) (Figure 1c). And simply by considering their number, it is difficult to believe that these thousands of molecules all originate from the very same precursor material and were formed along one defined pathway.

(ii) The remarkable agreement between isolates of different origin (three of which are shown in Figure 4) makes this even more unlikely as it would require that the same precursor material would dominate each of the environments, and the same limited number of transformation reactions would have occurred. Considering the different location, climate, and compartment from which the river, the peat,

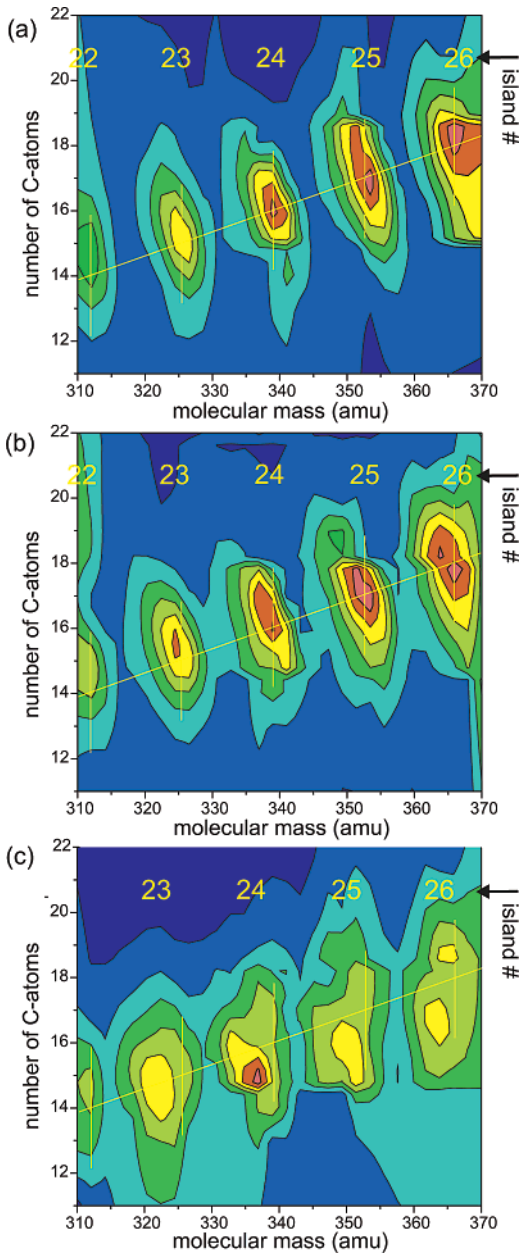

FIGURE 4. (a) Enlarged section of Figure 1e for the mass range *m/z* 310−370 of the river isolate. (b) Plot for the fulvic acid molecules of a Nordic tarn isolate. (c) Plot for the fulvic acids of a peat elutriate. Note that all three isolates show ≥ = 80% congruence in the molecular formulas that occur. The vertical and one diagonal lines mark the intensity maxima of the river isolate mass spectrum (Figure 1c).

and the tarn fulvic acids were isolated, this does not seem very likely. However, we cannot rule out that the same precursor materials of higher plant origin was present in these three locations, and it is not questioned that such material can be important as precursor of fulvic acids. Still, considering that this compositional agreement is true for more than one thousand molecules (probably for several thousand) that all follow the very same pattern remains remarkable. Moreover, a very high degree of agreement has recently been recognized by FTICR−MS analyses between DOM molecules of terrigeous and marine sources (*9*).

Potential Origin of Regularities. Besides being unlikely it is also not necessary to assume that the formation of fulvic acids proceeds along a defined pathway from one defined precursor material. The predictions made here by utilizing the structure information provided by tandem mass spectrometry (*3*, *8*, *10*, *11*) clearly show that the regularity in the elemental pattern of carboxylic acids found in fulvic acid isolates can be explained solely from their paucity of functional groups and the valency of the three elements from which these molecules are formed. The validity of our elemental prediction was confirmed by the fact that the overwhelming majority of molecules detected in the fulvic acid isolates actually fell within the predicted range. One defined precursor material is not required.

If this regularity in the molecules stems from the paucity of functional groups the question needs to be answered why fulvic acid isolates are so poor in functional groups and are dominated by (poly-)carboxylic acids with a limited number of hydroxy groups. It was previously suggested that in the course of oxidative alteration of very diverse source materials, all functional groups that can be hydrolyzed will be hydrolyzed, erasing functional groups such as ethers, esters, and lactones (*13*). Hydrolysis and oxidation, including oxidative cleavage of aromatic rings, are characteristic processes for the oxidative degradation of many biopolymers, e.g., of lignin (*20*). The combination of such reactions allows structurally diverse and complex molecules to converge gradually into the comparatively homogeneous set of fulvic acids, with simpler carbon skeletons and a strongly diminished diversity of functional groups (*13*).

Environmental Modulation. However, on the basis of the compositional fundament provided by the above processes the actual composition of a fulvic isolate may differ one from another in the way that more or less aromatic or more or less carboxylated molecules are more prominent. It is assumed that these relative differences either reflect properties of the precursor material or the regime under which its transformation into fulvic acids occurred.

For example the molecules found in the river isolate did not occupy the whole chemically allowed range in the C− vs mass-diagram (Figure 3), because environmental conditions narrow down the range of molecules actually found in a sample. For this isolate of an aqueous sample it is reasonable to assume that molecules situated in the top region of the calculated islands (low oxygen content) as well as in the very right section (high degree of saturation) of each of the islands will not be relevant due to their limited aqueous solubility. In this way environmental constraints transform the predicted triangles into ellipsoid islands (Figure 3).

Environmental modulations may also explain the relative intensity differences in the three isolates compared in Figure 4. A shift in the horizontal position of the intensity maxima indicates a different preferential degree of saturation. For the peat elutriate (Figure 4c), intensity maxima of all islands are clearly shifted toward the left, as compared to the river and tarn isolate (Figure 4a,b), indicating that unsaturated, potentially aromatic molecules are more frequent, and they exhibit a lower mass at a given C-number. This finding is consistent with the higher average degree of aromaticity in the peat elutriate previously detected by ^{13}C NMR spectroscopy (*2*; reproduced in ref *13*). As far as the frequency of carboxylate groups is concerned, ^{13}C NMR data do not show too much difference between the three samples. For the mass range shown in Figure 4, however, the intensity maxima in the mass spectra of the peat isolate (Figure 4c) appear at slightly lower C-numbers, indicating a higher relative contribution of more oxygenated molecules as compared to the two other isolates (Figure 4a,b). In this respect ^{13}C NMR data and high-resolution mass spectra do not agree. Given the fundamental differences in the analytical approaches of NMR and MS, it is not self-evident that both techniques yield consistent results. While ^{13}C NMR provides information on average properties of carbons in a fulvic acid isolate, FTICR−

MS detects relative signal intensities of large numbers of individual molecular species.

Fulvic Acids as Transient Organic Matter. These mass spectrometric results concerning the composition of molecules in the fulvic acid fraction are consistent with the concept that fulvic acid molecules are not the product of a specific (bio-) chemical (trans-)formation process of one certain class of biopolymers but an inevitable state of transition of organic material of different sources on its way to carbon dioxide. The organic precursor material may either be of biogenic (lignin (*10*), terpenoids (*11*), tannins, etc.) or geogenic (e.g., black carbon (*18*)) origin or may originate from abiotic condensation reactions. By hydrolysis and oxidation reactions, the precursor material becomes increasingly homogeneous in terms of its functional groups and enters the fulvic acid fraction. A similar nonspecific formation process by so-called geochemical reactions has been proposed before to explain the log-normal molecular weight distribution of fulvic acids (*21*).

Necessarily, these forces driving the formation of fulvic acids continue. Depending on their molecular composition and structure (Figure 2b) fulvic acid molecules will differ in their stability against further oxidative transformation. Enzymatic attack may be hindered by a highly branched aliphatic skeleton or the electrophilic attack of an aromatic system may be hindered by a high number of carboxylate groups (Figure 2b). Using high-resolution mass spectrometry it was recently shown that the reactivity of specific fulvic acid molecules toward ozone can be linked to their elemental composition and such structural features (*22*).

Less stable fulvic acids molecules will, thus, leave the fulvic acid state by ongoing oxidation that finally results in scission of their carbon skeleton and, eventually, end in carbon dioxide. Other molecules, whose structure hinders their further oxidation for kinetic reasons, may reside in the fulvic acid fraction for longer periods of time. The composition of one fulvic acid isolate is, thus, the combination of the hydrolytic and oxidative processes that lead to the formation of fulvic acids and those reactions that further oxidize fulvic acids. Kinetically stabilized, metastable molecules can be expected to dominate the composition of a fulvic acid fraction.

This view on fulvic acids as being a transient state may close a gap in the transformation pathways of many classes of biogenic compounds in the natural environment. On one hand primary oxidation, transformation, or degradation products of many compound classes, like lignin (*20*), terpenoids (*23*), or carotenoids (*24*), are known. These known products already tend to be of lower molecular mass and structurally less complex than their parent compounds, but their degree of oxidation is limited. On the other hand, their more polar and more oxidized transformation products are still largely unknown. Fulvic acids appear to be the suitable candidates to fill this gap.

Future investigations using FTICR−MS may improve our understanding of the conditions under which organic carbon is transformed into and transiently stored as fulvic acids and of the factors that foster the further oxidation of fulvic acids into more polar natural organic matter and, eventually, the release of carbon dioxide from this organic carbon pool.

Acknowledgments

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