Reports

Lignin Signature of Aquatic Humic Substances

Abstract. *Lignin-derived phenols dominate the cupric oxide oxidation products of dissolved humic substances from river and lake waters. The relative distributions of these phenols suggest the presence of intact, though oxidized, lignin, which is indicative of the locally dominant vascular plant vegetation. Recognizable lignin is present mostly in humic acid as opposed to fulvic acid fractions. This lignin component represents a source-specific and process-dependent tracer that can uniquely characterize dissolved organic matter.*

Dissolved organic matter (DOM) is a ubiquitous and dynamic component of lakes, rivers, and oceans (*1*). The composition of DOM is determined by both local biological sources and dominant microbial, geochemical, and physical processes occurring in the aquatic and surrounding environments. The diversity of potential sources and processes suggests that a characteristic organic chemical signature may exist for individual waters (*2*). However, a component of DOM has not yet been identified that reflects both biological sources and transformational processes.

A major and readily isolatable component of DOM in rivers and lakes is aquatic humic substances (*3*). Chemical analyses of these substances have revealed biochemical components including proteins (*4*), carbohydrates (*5*), and lignin structural units (*6, 7*). We have used CuO oxidation products of lignin in sedimentary organic matter as molecular tracers of vascular plant sources (*8*) and diagenetic histories (*9*). Here we apply the same technique (*10*) to aquatic humic substances to obtain information on (i) the relative contribution of vascular plant carbon and thus terrestrial carbon to dissolved humic substances, (ii) the type of vascular plant source material, and (iii) the degree of oxidative degradation of the original plant material.

Lignins are polyphenolic polymers found only in vascular plants (*11*). Alkaline CuO oxidation converts lignin into simple phenols whose chemical substitution patterns indicate an unambiguous lignin source and whose relative distributions reflect the type of vascular plant tissues (*8, 12*). To facilitate plant source identification, we group these phenols into families, based on their chemical substitution patterns (Fig. 1) (*13*). Thus, vanillyl phenols occur as oxidation products of all four categories of vascular plants (woods and nonwoody tissues of angiosperms and gymnosperms); syringyl phenols are produced only from angiosperm lignin and cinnamyl phenols only from nonwoody tissues (*8*).

To test this molecular tracer technique on dissolved humic substances, we chose two aquatic environments of distinctly different vegetation. Cullaby Lake (CL) is a brown-water coastal lake in western Oregon that receives drainage from a coniferous forest. Williamson River (WR) is a highly colored stream draining Klamath Marsh (southern Oregon) whose vegetation consists of marsh grasses and reeds (*4, 5*).

Water samples were centrifuged, acidified to *p*H 1.8, and passed through a column of prewashed XAD-7 resin. The adsorbed humic substances were eluted with aqueous triethylamine (0.15*M*), desalted on cation-exchange resin, and lyophilized (*14*). To separate humic acid and fulvic acid fractions, we dissolved the humic substances in 0.1*N* NaOH and 0.1*M* $Na_4P_2O_7$ and acidified to *p*H 1 (*15*). Humic acid precipitates were centrifuged and freeze-dried. For lignin analysis we reacted 20 mg of humic material with alkaline CuO for 3 hours at 170°C to produce simple phenols that were analyzed as trimethylsilyl derivatives by gas chromatography and identified by gas chromatography–mass spectrometry (*10*).

Lignin-derived phenols dominate the CuO oxidation products of WR and CL humic substances (Fig. 1). The total yields of lignin phenols normalized to organic carbon are high for these samples (Λ = 2.5 to 3.4 in Table 1) compared to the yields for humic material extracted from soils (Λ = 0.5 to 1) and nearshore marine sediments (Λ = 1 to 2) (*16*). Because lignin is found only in vascular plants, this implies that a significant portion of aquatic humic substances is de-

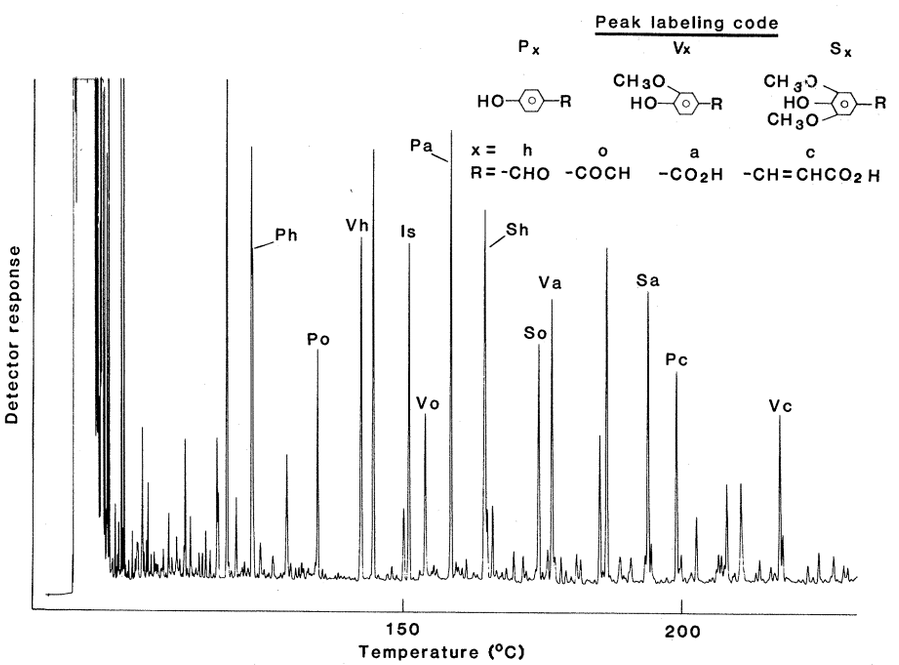

Fig. 1. Gas chromatographic trace of CuO oxidation products of Williamson River humic substance as trimethylsilyl derivatives. Chromatographic conditions are described in (*10*). Peak identification (as unsilylated precursors) is as follows: *Ph*, *p*-hydroxybenzaldehyde; *Po*, *p*-hydroxyacetophenone; *Vh*, vanillin; *Is*, ethylvanillin (gas chromatographic internal standard); *Vo*, acetovanillon; *Pa*, *p*-hydroxybenzoic acid; *Sh*, syringaldehyde; *So*, acetosyringone; *Va*, vanillic acid; *Sa*, syringic acid; *Pc*, *p*-coumaric acid; and *Vc*, ferulic acid.

Table 1. Lignin-derived phenol compositions for total humic substances (HS), humic acids (HA), and fulvic acids (FA); *S/V* is the number of milligrams of syringyl (*S*) phenols (*Sh*, *So*, and *Sa*) divided by the number of milligrams of vanillyl (*V*) phenols (*Vh*, *Vo*, and *Va*); *C/V* is the number of milligrams of cinnamyl (*C*) phenols (*Pc* and *Vc*) divided by *V*; *P/V* is the number of milligrams of *p*-hydroxyl (*P*) phenols (*Ph*, *Po*, and *Pa*) divided by *V*; Λ is the sum of *V*, *S*, and *C* normalized to 100 mg of organic carbon in the sample (*10*). The last three columns are weight ratios of individual phenols. Abbreviations are explained in Fig. 1.

Component	Organic carbon (%)	*S/V*	*C/V*	*P/V*	Λ	*Va/Vh*	*Sa/Sh*	*Pc/Vc*
			Cullaby Lake					
HS	44.6	0.23	0.051	0.33	2.49	0.98	0.54	0.85
HA	49.4	0.28	0.026	0.19	4.17	0.88	0.42	0.63
FA		0.028	0.088	0.49	1.04	1.3	1.1	4.5
			Williamson River					
HS	48.1	1.6	0.47	1.0	3.40	0.79	0.63	0.95
HA	51.5	1.8	0.41	0.82	5.20	0.75	0.54	1.1
FA		0.026	0.16	1.6	0.82	1.2	0.86	4.6

rived from terrestrial plant sources by direct leaching of plant material (*17*) or mobilization of soil organic matter. If lignin is present in humic substances as cross-linked phenylpropanoid units as in plants, then approximately 5 percent of the humic carbon is contained in lignin structural units (*18*). However, 5 percent is a minimum estimate of the contribution of vascular plant carbon to aquatic humic substances since lignin represents only 5 to 30 percent (by weight) of vascular plant tissues (*11*). In fact, lignin concentrations in the WR and CL humic substances are greater than in many types of nonwoody vascular plant tissues (*8*); these results suggest that this tracer may be useful in environments where significant dilution by nonvascular carbon occurs.

Compositionally, the lignin components of the WR and CL humic substances are quite different. Compared to the CL humic substance, the WR sample is relatively enriched in syringyl and cinnamyl phenols (*S/V* and *C/V* ratios in Table 1). When plotted versus the compositional ranges of four common types of plant tissues (Fig. 2), the WR humic substance falls within the range of nonwoody angiosperm tissues, whereas the CL humic substance lies near the region of woody gymnosperm tissues. This finding is in good agreement with the known distribution of vascular plant vegetation in the respective drainage regions. Further evidence for this correspondence can be seen in the relative distribution of *p*-hydroxyl phenols (*P/V* in Table 1), which, although not exclusively lignin-derived, are produced by CuO oxidation of nonwoody angiosperm tissues and to a minor extent gymnosperm wood (*9*). Thus the high *P/V* ratio also is consistent with nonwoody angiosperm tissues as source material for the WR humic substance (Table 1).

Within the vanillyl and syringyl families the relative distribution of aldehydic, ketonic, and acidic oxidation products is extremely constant from vascular plant tissues (*9*) and appears to be related to characteristic linkages of the propanoid side chains (*11*). The production of all three phenolic forms from aquatic humic substances indicates that lignin in these substances is present in polymeric form similar to that found in vascular plants. However, the relative yields of individual phenols are consistently different from the yields for plants. The acid/aldehyde ratios of plant tissues are 0.15 ± 0.05 for vanillyl and syringyl phenols (*9*), whereas both of our humic substances have acid/aldehyde ratios of 0.79 to 0.98 for vanillyl phenols and 0.54 to 0.63 for syringyl phenols (*Va/Vh* and *Sa/Sh* in Table 1). These results suggest that the lignin components of these humic substances have undergone oxidative degradation and could explain how relatively insoluble lignin might be incorporated into dissolved humic substances. Though modified, these humic substances retain sufficient lignin signature to indicate the general type of vascular plant source material.

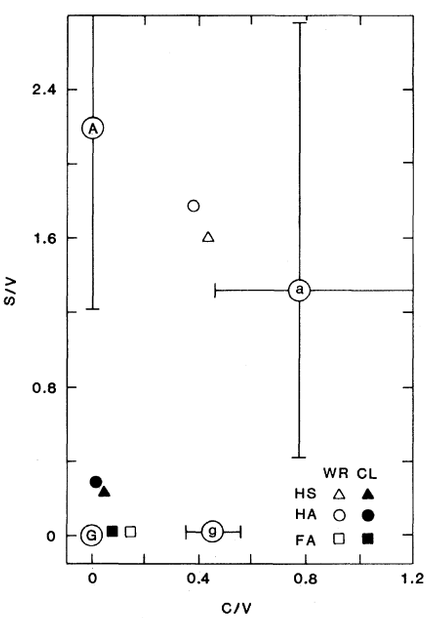

Fig. 2. Lignin compositional plot of Cullaby Lake (*CL*) and Williamson River (*WR*) humic acids (*HA*), fulvic acids (*FA*), and total humic substances (*HS*). Also included are the corresponding means and ranges for four types of vascular plant tissue: gymnosperm woods (*G*), nonwoody gymnosperm tissues (*g*), angiosperm woods (*A*), and nonwoody angiosperm tissues (*a*). Plant data are from (*8*).

Humic substances consist of a range of acidic components that can be partitioned into acid-soluble fractions (fulvic acids) and acid-insoluble fractions (humic acids). The WR humic substance is composed of equal portions of humic and fulvic acids, whereas the CL sample is two-thirds fulvic acids. In both samples we found that humic acids produce four to six times more lignin phenols relative to organic carbon than fulvic acids and compositionally resemble the local vegetation patterns. The significantly higher syringyl (*S/V*) yields and lower *p*-hydroxyl phenol (*P/V*) yields for humic acids as compared to corresponding fulvic acids indicate a concentration of methoxylated lignin structures in the humic acid fractions. This pattern is also present within the cinnamyl phenols. In addition, humic acids yield consistently lower acid/aldehyde ratios than fulvic acids, as also have been observed with sedimentary humic substances (*16*).

The lignin components of fulvic acids differ from those of humic acids in a direction expected to result from microbial alteration. Aerobic fungal degradation of lignin results in increased acidity and methoxyl demethylation (*19*) with the eventual loss of the characteristic lignin signature (*20*). Although the lignin components of the total humic substances are degraded relative to vascular plant lignin, it is not clear whether the differences between humic and fulvic acids are due to diagenesis or are a function of the partitioning process. However, it is clear that the lignin components of humic acids could be transformed into those of fulvic acids by demethylation and oxidation but not vice versa.

We believe that lignin analysis by CuO oxidation has potential applications for characterizing aquatic humic substances, a major component of DOM. For example, because lignin is exclusively a component of terrestrial plants, lignin analysis could be used to distinguish allochthonous from autochthonous inputs of DOM with the high sensitivity of

a molecular tracer (*21*). Specific questions concerning the seasonal input of DOM that might result from either phytoplankton productivity or litterfall and increased leaching could be evaluated by this technique. Moreover, the degree of oxidation of the lignin signature might be used to distinguish inputs of soil organic matter from fresh leachate. For river systems draining different vegetative regions, lignin analysis could reveal the geographic sources of DOM by identification of the regionally characteristic vascular plant sources. Also, differences in the lignin signature of humic and fulvic acids could be exploited to determine their relative conservation during estuarine mixing (*22*). Finally, the production of lignin-derived phenols from the CuO oxidation of marine humic substances would be an unambiguous indicator that terrestrially derived carbon is present in the marine dissolved organic pool (*23*).

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Human Proto-Oncogene Nucleotide Sequences Corresponding to the Transforming Region of Simian Sarcoma Virus

Abstract. *The nucleotide sequences of the six regions within the normal human cellular locus (c-sis) that correspond to the entire transforming region of the simian sarcoma virus (SSV) genome (v-sis) were determined. The regions are bounded by acceptor and donor splice sites and, except for region 6, resemble exons. Region 6 lacks a 3′ donor splice site and terminates −5 base pairs from the 3′ v-sis-helper-viral junction. This is consistent with a model proposing that SSV was generated by recombination between proviral DNA of simian sarcoma associated virus and proto-sis and that introns were spliced out subsequently from a fused viral-sis messenger RNA. This also suggests that the 3′ recombination occurred within an exon of the woolly monkey (Lagothrix) genome. The open reading frames predicting the v-sis and c-sis gene products coincide with the stop codon of c-sis located 123 nucleotides into the fifth region of homology. The overall nucleotide homology was 91 percent with substitutions mainly in the third codon positions within the open reading frame and with greatest divergence within the untranslated 3′ portion of the sequences. The predicted protein products for v-sis and c-sis are 93 percent homologous. The predicted c-sis gene product is identical in 31 of 31 amino acids to one of the published sequences of platelet-derived growth factor. Thus, c-sis encodes one chain of human platelet-derived growth factor.*

The simian sarcoma virus (SSV) is the only acutely transforming retrovirus (causing disease within a short latency period) that has been obtained from primates. After isolation of this virus from a fibrosarcoma of a pet woolly monkey (*Lagothrix*) (*1*), the molecularly cloned genome was shown to contain 1.0 kilobase (kb) of woolly monkey–derived cellular sequences that are responsible for the transforming potential of the virus (*2–4*). Since SSV is related to the nonacutely transforming gibbon ape leukemia viruses, infection of the woolly monkey with a virus from this group probably preceded the recombination event giving rise to SSV (*5*).

The complete nucleotide sequence of the viral transforming gene as well as immunological data have shown that the transforming product of SSV is a 28,000 dalton protein, $p28^{sis}$, that is probably encoded mostly by sequences derived from woolly monkey cellular DNA (*6*). Several amino acid residues at the amino terminus of p28 are thought to be coded by the envelope (*env*) gene of the helper virus derived sequences.

The human cellular homolog of the SSV genome, c-*sis*, contains 1.0 kb of coding regions, distributed over 12.5 kb, that are interspersed by at least four introns (*7*). *Sis*-related messenger RNA's (mRNA's) of 4.2 kb can be detected in sarcomas, glioblastomas, and some human T-cell leukemia virus (HTLV)–infected cell lines (*8*). These results and the nucleotide sequence data of the 5′ cellular (c-*sis*) gene indicate that additional exons must be present 5′ and may also be present 3′ to the v-*sis* region of homology (*9*).

Recent sequence comparisons indicated that the predicted v-*sis* transforming product is highly homologous to human platelet-derived growth factor (PDGF) (*10, 11*). It is likely that most of the sequence differences are species specific; however, it is not known whether additional changes could have occurred which give v-*sis* its transforming potential. Although v-*sis* is known to transform mouse fibroblasts in vitro (*8*), addition of PDGF to the cells does not cause morphological transformation (*12*). Thus, it is not known whether transformation is due to intracellular expression of a functionally normal or altered *sis* gene product. Transformed cells of mesenchymal origin that express the *sis* message correlate well with tissues known to be mitogenically stimulated by PDGF, that is, fibroblasts and glial cells (*8, 13*). The restricted expression of *sis* in a large