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Binding of Polychlorinated Biphenyls to Aquatic Humic Substances: The Role of Substrate and Sorbate Properties on Partitioning

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Two ortho- (2,2′,5 and 2,2′,5,6′) and a non-ortho- (3,3′,4,4′) substituted polychlorinated biphenyl (PCB) congeners were used to study the effects of sorbate structure in binding processes to two lacustrine fulvic acids. Binding constants were determined by solubility enhancement of the solutes by the fulvic acids. The binding of the ortho-trichlorobiphenyl was significantly less than the non-ortho-substituted tetrachlorobiphenyl to both fulvic acids. Surprisingly, the measured ortho-trichlorobiphenyl binding constant to both fulvic acids was approximately the same as the ortho-substituted tetrachlorobiphenyl. The effect of the chlorines in the ortho position inhibits free rotation around the 1,1′ carbon bond, thereby making the molecule less able to interact effectively with the fulvic acid substrate relative to its non-ortho-substituted congeners. Finally, binding of all three PCBs to the Great Dismal Swamp fulvic acid was significantly higher than for the Pony Lake sample. This observation is attributable to the former substrate's higher degree of aromaticity and polarizability, which can potentially interact more favorably with the PCBs through an increase in van der Waals type interactions.

Introduction

The speciation of hydrophobic organic contaminants (HOCs) in natural waters is affected by the presence of humic substances. Partitioning of HOCs to humic and fulvic acids increases their apparent aqueous-phase concentration and may alter their reactivity (sorption, bioavailability, transformation) in the environment. Because dissolved humic materials are ubiquitous to natural waters, accurate determinations of the pollutant−humic substance (quantified as dissolved organic carbon) partition coefficients (K_{doc}) are of paramount importance in assessing the fate and transport of HOCs in aqueous systems.

Over the past several years, analytical techniques have been developed to measure the humic substance−HOC partition coefficient (*1*−*10*), including fluorescence quenching (*4, 6*−*8*), reverse-phase column techniques (*5, 9*), dialysis equilibrium (*1, 11*), and aqueous solubility enhancement (*2, 3, 12*). The application of the fluorescence quenching technique is complicated by the fact that many HOCs do not possess fluorophores, and some controversy has recently evolved over the mechanisms responsible for this phenomenon (*8*). The reverse-phase column method may not be accurate for measuring K_{doc} because of nonequilibrium effects, while the use of dialysis tubing is limited to those humic materials with molecular weights significantly larger than the membrane poresize. The solubility enhancement method, however, has yielded consistent results and is not plagued by many of the analytical problems associated with the other methods (*2, 3*). One limitation of this approach is that it requires a large amount of humic material, but this technique was modified to reduce the volume of the humic solution to 8 mL for each experiment.

The binding of HOCs to humic substances had at first been assumed to be a nonspecific process whereby the magnitude of K_{doc} is roughly proportional to the contaminants' 1-octanol−water partition coefficient (K_{ow}) or aqueous activity coefficient (γ_w) (*2, 12*). Recent studies have suggested that the structure of both the humic material and the HOC may control binding reactions (*3, 11, 13*−*15*). In a previous paper, we observed unusually large K_{doc} values for pyrene's association with highly aromatic (>20%) humic materials (*12*). We attributed this process to a combination of favorable molecular interactions between the analyte and the humic aromatic moieties coupled with pyrene's planar configuration that allows it to be less sterically hindered in the humic matrix than other bulkier HOCs (*12*).

In this paper, we test the hypothesis that both steric hindrance and molecular interactions between HOCs and aromatic moieties of humic substances may control HOC−humic material binding reactions. We selected a non-ortho-substituted (3,3′,4,4′) and two ortho-substituted (2,2′,5′ and 2,2′,5,6′) polychlorinated biphenyl (PCB) congeners to probe HOC binding interactions in fulvic acids isolated from the Great Dismal Swamp in Virginia (a terrestrially derived humic substance) and Pony Lake in Antarctica (a humic material comprised entirely of phytoplankton organic matter) using a modified version of the solubility enhancement method. The non-ortho-substituted congener was selected because it has less hindered rotation around the carbon bond connecting the two phenyl rings (while not truly planar, it will be referred to as "coplanar" in this paper), while the ortho-substituted PCBs are constrained by the presence of the chlorines, which would inhibit free rotation of the rings (referred to as "nonplanar" in this paper). These experiments enable us to (i) determine how sorbate properties affect binding to humic substances in terms of steric effects and van der Waals interactions and (ii) investigate how humic materials with differing structural characteristics are able to influence the partitioning of hydrophobic organic contaminants.

Materials and Methods

The PCB congeners were purchased from Chemical Services (99+%) and were used without further purification. The fulvic acids were isolated using XAD-8 chromatography from the Great Dismal Swamp, VA, according to the methods described by Thurman and Malcolm (*16*). The fulvic acid from Pony Lake, Antarctica, was isolated using the same method and is identical to the material used by McKnight and co-workers (*18*). To evaluate the solubility enhancement of PCB congeners in the presence of humic materials, we employed a

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TABLE 1. K_{ow} Values for Selected PCBs and K_{doc} Values for PCBs

PCB	Pony Lake K_{doc} (L/kg of OC) ± 95% CI	Great Dismal Swamp K_{doc} (L/kg of OC)	aqueous solubility[a] (mol/L)	log K_{ow}[b]
2,2′,5	4620 ± 720	14 400 ± 3700	1.98×10^{-6}	4.73± 0.72; $n = 9$
2,2′,5,6′	4270 ± 400	14 300 ± 2200	1.63×10^{-7}	5.46; $n = 1$
3,3′,4,4′	38 000 ± 5800	52 300 ± 7500	1.87×10^{-9}	6.23 ± 0.44; $n = 5$

[a] Measured values taken from ref *25.* [b] Data taken from ref *26* and do not include any values that were "estimated" from LFERs.

modified version of the solubility enhancement method originally developed by Chiou and co-workers (*2, 3*). PCB congeners dissolved in hexane were plated onto the walls of 8-mL glass vials at concentrations well above their reported aqueous solubilities (×10) and in the absence of any drying gases. This procedure was followed to minimize volatile loss of the analytes because we observed losses of up to 30% of the PCB congeners when the vials were dried under a stream of argon gas. Buffered aqueous solutions (1 mmol L^{-1} KH_2PO_4 and 1 mmol L^{-1} K_2HPO_4) comprised of various concentrations of humic material (0−100 mg L^{-1} organic carbon) were added to each vial so that no headspace existed. The vials were covered with foil, and a Teflon-lined syringe cap was placed on each vial. The vials were allowed to equilibrate on a reciprocating shaker for several days. Upon equilibration, an aliquot of each vial was transferred to an autosampler vial using a 5-mL barrel syringe. All aliquots of the solutions were weighed, and equal amounts of hexane were subsequently added to each autosampler vial to ensure a 1:1 (v/v) extraction. Quantitative analysis of each congener was determined by GC using a Fisons 8360 GC equipped with an ECD and an automated cold on-column injector with helium as the carrier gas. The injection syringe was configured such that only the hexane layer was assayed. A fused silica column (RTX-5 Restek 15 m × 0.25 mm i.d.) was used to separate the PCB congeners with the oven temperature programmed from 37 °C for 1 min to 100 °C at 20 °C/min. The temperature was held isothermally at 100 °C for 1 min, increased from 100 to 300 °C at 5 °C/min, and finally held at 300 °C for 20 min. Subsets of humic solutions were assayed by total organic carbon analysis (Shimadzu TOC 5000) to determine the precise concentrations of humic material in each vial.

Solid-state cross polarization magic-angle-spin (CPMAS) ^{13}C NMR spectra were measured on a 200 MHz Chemagnetics CMX spectrometer with a 7.5 mm diameter probe. Spectra were measured on a 100 mg isolated sample. All experiments were performed with a spinning rate of 5000 Hz, a pulse delay of 1 s, a pulse width of 4.5 μS for the 90f pulse, and a contact time of 1 mS. A line broadening of 100 Hz was applied in the Fourier transformation of the free induction decay data.

Results and Discussion

Apparent aqueous solubilities of selected PCBs were measured in solutions containing fulvic acids isolated from two distinct aquatic environments, Great Dismal Swamp, VA, and Pony Lake, Ross Island, Antarctica. Dissolved organic carbon (DOC) concentrations of these solutions ranged from 0 (blank) to 100 mg/L DOC. We fitted our solubility enhancement data to the equation derived by Chiou and co-workers (*2*):

$$S_w^*/S_w = 1 + K_{doc}[HS] \qquad (1)$$

where S_w^* and S_w are the respective solubility of each PCB congener in the presence and absence of humic materials and [HS] is the humic substance concentration expressed as dissolved organic carbon (DOC kg L^{-1}). Results of the solubility enhancement experiments of non-ortho-substituted (3,3′,4,4′) and ortho-substituted (2,2′,5′ and 2,2′,5,6′) PCBs showed that the apparent solubilities of each PCB increases with increasing DOC concentration for both substrates (Figures 1 and 2). The K_{doc} values for the PCBs bound to the Pony Lake fulvic acid ranged from 4270 to 38 000 L/kg with 3,3′,4,4′-PCB possessing the highest value (Table 1). Similarly, this congener possessed the highest PCB K_{doc} value for the GDS fulvic acid, and the K_{doc} for all three congeners were up to a factor of 3 times higher for this humic material (Table 1).

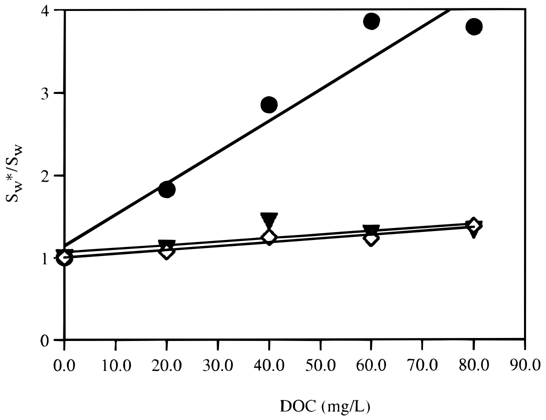

FIGURE 1. Binding of PCBs by Pony Lake fulvic acid as determined by the solubility enhancement method (error bars are within font size): (◊) 2,2′,5-PCB, (▼) 2,2′,5,6′-PCB, (●) 3,3′,4,4′-PCB.

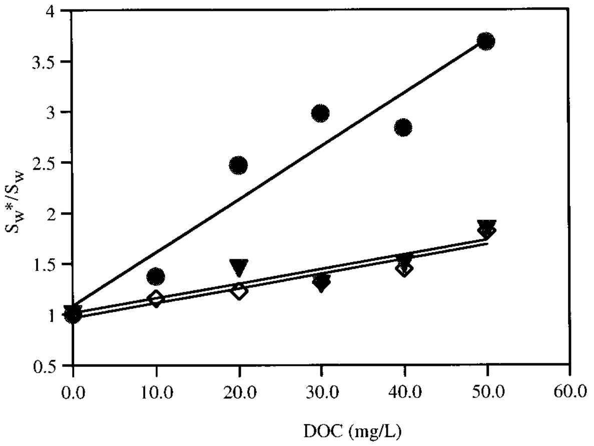

FIGURE 2. Binding of PCBs by Great Dismal Swamp fulvic acid as determined by the solubility enhancement method (error bars are within font size): (▼) 2,2′,5,6′-PCB, (◊) 2,2′,5-PCB, (●) 3,3′,4,4′-PCB.

The humic substances used in this study were chosen because they represent humic materials from two geochemical end member environments (i.e., with and without lignin as precursor organic material) and exhibit widely different physical and chemical properties (*17*). Pony Lake (PL) fulvic acid was recovered from a small Antarctic lake where the only sources of organic matter in the water column are phytoplankton (mostly *Chlamydomonas intermedia* and *Cryptomonas* sp.) and bacteria (*18*). The Great Dismal Swamp

(GDS) fulvic acid is predominantly derived from decayed higher plant debris. Based upon our ^{13}C NMR data, Pony Lake fulvic acid contains a higher percentage of aliphatic I (sp^3 bonds) carbon (50.6%) in comparison to the Great Dismal Swamp fulvic acid (41.9%). Typically, humic substances derived from solely algal and microbial sources reflect this origin and are more aliphatic in character. In terms of the percentage of aromatic moieties (as measured by ^{13}C NMR), GDS fulvic acid has a higher percentage (19.7%) than the PL fulvic acid (12.7%). Thus, the presence of lignin as a precursor organic material influences the chemistry of the GDS fulvic acid by contributing to its aromatic character.

In a previous study (*12*), we observed a strong correlation between the humic substrates aromaticity and its ability to partition pyrene, a four-ring polycyclic aromatic hydrocarbon (PAH). We report a similar result in this paper for the PCB congeners where our analytes interact more favorably with the GDS fulvic acid relative to the less aromatic PL isolate. One reason for this preferential interaction is the increased polarizability of the substrate in aromatic-rich humic substances (*12, 13*). An increase in the polarizability of the humic material will result in an increase in van der Waals interactions between the solute and substrate. In an aromatic-rich environment such as the GDS fulvic acid, PCBs would be particularly susceptible to these interactions for those congeners that possess weak dipole moments. Conversely, the Pony Lake fulvic acid is significantly less aromatic and would presumably be less polarizable. Thus, as observed in our earlier work for pyrene (*12*), the magnitude of the PCB K_{doc} values is dependent upon the aromatic nature of the fulvic acid substrate (Table 1). Overall, microbially and algal derived types of humic materials appear to be less capable of binding aromatic HOCs relative to fulvic acids derived from terrestrial sources (*12*).

Past studies have attributed differences in a humic material's ability to partition HOCs to its polarity index based upon the relative abundance of oxygen and nitrogen in the humic material's structure. This approach is valid when comparing humic materials with significantly different oxygen contents (>10's of percent). Typically these relationships exist for humic substances derived from very different environments, e.g., commercial vs terrestrial vs aquatic sources, and isolated by different methods, e.g., XAD vs ultrafiltration. The two aquatic fulvic acids used in this study were all isolated in an identical manner using a preparative liquid chromatography column packed with a hydrophobic XAD-8 resin. Thus, the stationary phase capacity factor (which roughly quantifies the polarity of the material) should be identical for both the GDS and PL fulvic acids, and we would expect little differences in the overall polarity of both samples. While oxygen contents were not measured for these samples (due to a lack of material), our previous paper (*12*) observed only very small differences in the oxygen content (less than a few percent) for a diverse number of aquatic fulvic acids isolated by XAD-8 chromatography. Thus, we believe that while both fulvic acids possess relatively similar polarities (because they were isolated using the identical hydrophobic resin), their structural characteristics are sufficiently different and would explain their different partitioning characteristics to the PCB congeners.

Studies have shown a relationship between K_{doc} and K_{ow} such that compounds which exhibit relatively high values of K_{ow} have higher values of K_{doc} (*2, 19*). This observation has largely been attributed to the hydrophobic nature of many organic contaminants. Thus, large molecules possessing lower aqueous solubilities would bind more strongly to the humic substrate (*3, 5, 11*). For example, the addition of chlorine atoms to the parent structure of PCBs (i.e., biphenyl) would presumably increase its hydrophobicity in an additive manner, and K_{doc} values for these substances should be greatest for those congeners with the largest number of Cl^- substituents (*19*). One exception to this rule is substitution of chlorines in the ortho position of the biphenyl ring (*24*). As stated earlier, chlorines in the ortho position would restrict rotation around the 1,1′ carbon bond. Typically these congeners possess smaller K_{ow} values than their non-ortho-substituted isomers (Table 1). Nonetheless, the hydrophobicity of both (ortho and non-ortho) tetrachlorobiphenyls (Table 1) are larger than the ortho-substituted trichlorobiphenyl, and a similar trend for their K_{doc} values should also exist if interactions between the analyte and humic material are controlled by simple dissolution phenomenon.

The ortho-substituted 2,2′,5,6′-tetrachlorobiphenyl exhibited much lower partitioning to both fulvic acids than the non-ortho-substituted, coplanar 3,3′,4,4′ isomer. While this is consistent with their K_{ow} values (Table 1), the ortho-substituted tetrachlorobiphenyl K_{doc} value is surprisingly the same (within the reported 95% confidence intervals) as that measured for the ortho-substituted trichorobiphenyl. Our observations suggest that those congeners capable of “free rotation” of the phenyl rings are able to interact more favorably with humic substances. In an earlier paper (*12*), we reported a K_{doc} for pyrene (a truly planar molecule) that was slightly smaller (about a factor of 2) than the value reported for DDT bound to the identical substrate (Aldrich humic acid). DDT is significantly more hydrophobic than pyrene (by more than a factor of 17 based upon their respective K_{ow} values) and possesses a three-dimensional configuration. We attributed our observations to the less favorable steric environment for the DDT molecule, which would affect its ability to interact favorably with the humic substrate. The ortho-chloro biphenyl congeners were chosen for this study in an attempt to demonstrate the effect of an out-of-plane position on partitioning phenomenon to specific humic substrates. Crystallographic evidence for 2,2′,4,4′,6,6′-PCB, which is comprised of four ortho-substituted chlorine atoms, shows that the dihedral angle between the mean planes of the two rings is constrained at 87.3° (*20*). As a consequence, the ortho-substituted PCBs would be less susceptible to polarization effects by the humic substrate. For example if humic−PCB binding interactions are analogous to their solubility in an organic solvent, then the solvent molecules would be able to access and interact with *all regions* of the solute molecule irrespective of it structural orientation, i.e., coplanar vs nonplanar. In a humic material, however, certain polarized functional groups are constrained to the carbon skeleton of the substrate and may not be able to induce a dipole moment in an ortho-PCB molecule as efficiently because they would be *unable to* access all areas of the sorbate. Conversely, the coplanar PCBs maybe more accessible to these polarized functional groups, due to their less restricted rotation around the 1,1′ carbon bond and as a result more capable of forming a van der Waals type molecular bond. Thus, irrespective of the humic substrate used in this study, the binding of the nonplanar tetrachlorobiphenyl congener was significantly less than the coplanar PCB isomer, and it was no stronger than the nonplanar trichlorobiphenyl. An analogous process has been observed in the bioavailabilty literature where phytoplankton bioaccumulation factors of four tetrachlorobiphenyls were up to 5 times higher for non-ortho-substituted PCBs relative to their equivalent ortho-substituted congeners (*21*). Finally, these steric effects may in part explain the increased toxicity of coplanar PCB congeners to fish and other organisms (*27, 28*) relative to their ortho-substituted isomers.

In conclusion, we observed that the binding of PCBs with different steric configurations impacts its ability to be bound by two fulvic acids. This behavior cannot be satisfactorily explained by dissolution-based partition models where the solvent phase is able to interact with all areas of the sorbate.

Moreover, the properties of the fulvic acid will impact the magnitude of the binding process, and it appears that those humic materials that are aromatic in nature are better able to bind HOCs. Finally, we believe that while simple partitioning relationships (i.e., octanol/water partition coefficients) are able to provide "ballpark" estimates of humic material−HOC binding constants, both specific substrate and sorbate properties must also be considered to obtain a more accurate value.

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