A "Site Creation" Model for Specific Adsorption of Aqueous Nucleobases, Nucleosides, and Nucleotides on Compost-Derived Humic Acid

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Aqueous nucleic acid constituent solutes cytosine (**1**), cytidine (**2**), cytidine 5′-monophosphate (**3**), thymine (**4**), thymidine (**5**), thymidine 5′-monophosphate (**6**), uracil (**7**), uridine (**8**), uridine 5′-monophosphate (**9**), adenine (**10**), adenosine (**11**), adenosine 5′-monophosphate (**12**), guanosine (**13**), and guanosine 5′-monophosphate (**14**) adsorb on compost-derived humic acid in sequential steps A, B, and C. Langmuir analysis of the isotherms gives a site capacity v_i and equilibrium constant K_i for a given solute and adsorption step i that increases $v_A < v_B < v_C$ and decreases $K_A > K_B > K_C$ at 25.0 °C. Data at six temperatures in the range 10.0−40.0 °C indicate that structurally related solutes like **7**, **8**, and **9** adsorb through their nucleobase units. Adsorption of a solute in step A, B, or C can be endothermic, thermoneutral, or exothermic. This paper uses the site capacity v_i at 25.0 °C, the average site capacity over the experimental temperature range $\langle v_i \rangle$, the adsorption equilibrium constant K_i at 25.0 °C, and the adsorption enthalpy ΔH_i and entropy ΔS_i for each solute to propose a model consisting of four components for each step i = A or B or C: (a) solute adsorption on a specific HA site A, B, or C involved in respective step A, B, or C; (b) as a result of primary adsorption on site A or B or C for many solutes, freeing up or "creation" of more sites A or B or C, respectively, from interactions A ... X, Y, etc. with other humic acid (HA) structural components X, Y, etc.; (c) solute adsorption at the newly available sites A or B or C; and (d) site hydration by water. The model accounts for the wide range of measured adsorption enthalpies and entropies. Among several conclusions is that adenine has an outstanding ability to interact with A and B sites of compost-derived HA and that B sites are harder for other solutes to access than A and C sites. Humic acids change their dimensions and state of aggregation to suit their environment. It now appears that selectivity for a solute is related to HA's ability to make more adsorption sites available near the point of primary solute interaction.

Introduction

We are interested in the sorptive[1,2] and other properties of humic substances (HS), which are heterogeneous biopolymers found in animals,[3] compost,[4] plants,[5] soils,[6,7] and sediments.[8] HS are nature's most ubiquitous water retainers, solute and metal sorbents, and system regulators.[6,7,9]

We are especially interested in a subset of HS called humic acids (HAs), which are insoluble in water at pH 1 and dissolve at pH's that vary with HA metal and mineral contents. HA molecules have a hydrophobic framework of aromatic rings linked by more flexible carbon chains and contain alcohol, amine, carboxylic, carbonyl, phenol, and quinone functional groups.[10,11] HA molecules extend at high pH because of their surface negative charge, but neutralization of their carboxylate and phenolate groups at lower pH causes aggregation to give HA "particles".[9]

Wershaw's membrane−micelle model[12] of aggregated HA molecules proposes a hydrophobic interior with polar hydrophilic functional groups (e.g. carboxylic acid) on the surface. Functional groups that are less polar because of hydrogen bonding and other interactions are in the interior. This model accounts for HA aggregation and attachment to clays and minerals through surface interactions. It describes sorption of hydrophobic organic compounds (HOCs) in terms of partition in the hydrophobic HA interior, as in solute distribution between water and an "organic liquid". Polar solutes such as pesticides also can interact with HA functional groups or more rigid structural features like stacked aromatic rings. Sorption of two solutes by partition is expected to be much less competitive than specific interaction with HA functional groups or rigid structural features.[13,14]

This paper models extensive data for adsorption of aqueous nucleic acid constituent solutes on a compost-derived HA.[1,2] These solutes are highly functionalized, hydrophilic, and polar. As such, they should interact much more specifically with HA than HOCs and most pesticides. We found that the isotherms can be analyzed with the Langmuir model for specific interaction with HA.[1,2] Our objectives are to interpret the data, compare the results with literature data for adsorption of other solutes on HA, and to offer a model for HA interactions with nucleic acid constituents that accounts for the observations, particularly the wide range of measured adsorption enthalpies and entropies. We conclude that primary adsorption of nucleic acid constituents on compost-derived HA sites can result in the freeing up or "creation" of more sites for solute adsorption.

General Observations

The adsorption data considered here were collected with a large, single sample of compost-derived HA. A thorough sequential solvent−aqueous base humic acid extraction procedure[15,16] was followed. The aqueous HA gel obtained at pH 1 was washed with water and vacuum oven dried (40 °C), and the resulting solid was thoroughly homogenized. Care was

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TABLE 1: Site Capacities for Adsorption of Nucleic Acid Constituents on HA[a]

	step					step			
solute[b]	A	B	C	$\sum\langle v\rangle$[c]	solute[d]	A	B	C	$\sum\langle v\rangle$[c]
cytosine, **1**	0.13	*e*	3.3		adenine, **10**	0.09	2.9	0.52	
	0.07	*e*	3.3	3.37		0.13	5.4	0.43	5.96
cytidine, **2**	0.03	*e*	0.2		adenosine, **11**	0.02	0.08	0.18	
	0.08	*e*	0.6	0.68		0.03	0.07	0.21	0.31
cytidine-p, **3**	0.05	*e*	0.26		adenosine-P, **12**	0.03	0.05	1.3	
	0.03	*e*	0.27	0.30		0.02	0.06	0.95	1.03
thymine, **4**	0.06	*e*	0.10		guanosine, **13**	0.03	0.12	1.2	
	0.06	*e*	0.08	0.14		0.03	0.12	1.1	1.25
thymidine, **5**	0.08	*e*	0.4		guanosine-P, **14**	0.02	0.07	0.16	
	0.08	*e*	0.28	0.36		0.04	0.06	0.19	0.29
thymidine-P, **6**	0.05	*e*	0.65						
	0.04	*e*	0.43	0.47					
uracil, **7**	0.01	0.05	0.1						
	0.01	0.04	0.35	0.40					
uridine, **8**	0.02	0.09	0.39[f]						
	0.01	0.05	0.06	0.12					
uridine-P, **9**	0.03	*e*	0.24						
	0.02	*e*	0.30	0.32					

[a] Units are mmol/g HA. The first entry for each solute is the measured v_i at 25.0 °C. The second entry is the average value $\langle v_i\rangle$ over the temperature range 10.0−40.0 °C. [b] Data from ref 1. [c] Total average site capacity, the sum of the preceding three columns. [d] Data from ref 2. [e] Step B not detected, see refs 1 and 2. [f] Data not used in calculating average $\langle v_i\rangle$ (see refs 1 and 2).

taken in its subsequent storage and use.[1,2] These are important points for the following reasons.

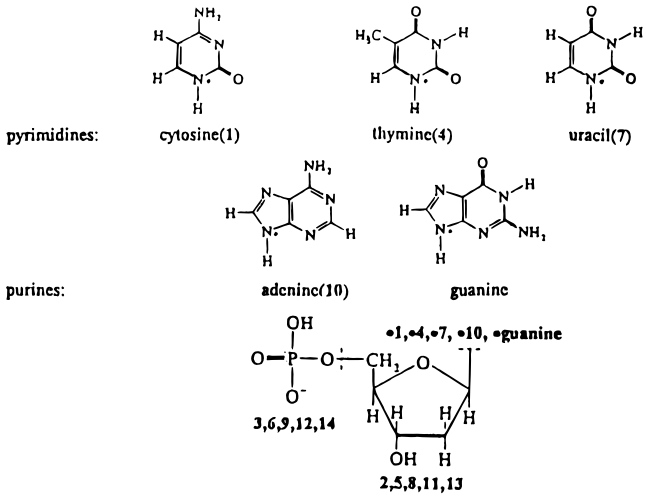
1. Molecules like benzene and adenosine 5′-monophosphate have unique molecular structures. Polystyrene has the same $(PhCHCH_2)_n$ building block in molecules of different molecular weight. HAs have neither of these characteristics. They have different primary or higher structures and exist as polymers whose building blocks, building block connectivity, functional groups, and molecular size can vary, depending on the HA source and its environment.[9−12]

2. The type, number per gram of HA, and distribution of the functional groups can depend on the HA source and sample.[9−11] Here we are thinking of the number of functional groups per gram of HA that are adsorption sites, if they are always the same (alcohol, amine, carbonyl, carboxyl acid, phenol, quinone),[6−11] whether they are present in the same relative proportions,[11] and how they are distributed on and in the material.

3. HAs are delicate materials that must be carefully extracted and treated. They are altered by too long of an exposure to strong acids or bases, which break them into more soluble fragments.[9,10,17] HAs should not be heated at temperatures much above 40 °C. Heating HAs decarboxylates their −COOH groups and causes irreversible changes in their other functional groups.[18,19] Functionality changes alter the adsorptive and other properties of HAs. They must be avoided to obtain reproducible data.

We studied solute adsorption over a wide concentration range, with many individual solute concentrations and HA aliquots, at six different temperatures.[1,2] This enabled us to (a) recognize the effects of occasional HA aliquot heterogeneity and of irreversible adsorption (for example, solubilization of HA with adenine); (b) establish the circumstances for reversible adsorption that leave HA insoluble; and (c) analyze the data for reversible systems with a thermodynamic, Langmuir model. This procedure revealed three sequential HA adsorption steps A, B, and C with increasing solute concentration and allowed us to measure the site capacity v_i, equilibrium constant K_i, and thermodynamic parameters ΔH_i and ΔS_i for each detectable adsorption step. We thus gained four quantities for each detected step. This gave up to 12 adsorption quantities for each solute. We also learned whether the HA capacity v_i for a fixed solute in a given step A, B, or C is temperature-dependent or not.[1,2]

We followed this procedure with the structurally related solutes cytosine (**1**), cytidine (**2**), cytidine 5′-monophosphate (**3**), thymine (**4**), thymidine (**5**), thymidine 5′-monophosphate (**6**), uracil (**7**), uridine (**8**), uridine 5′-monophosphate (**9**), adenine (**10**), adenosine (**11**), adenosine 5′-monophosphate (**12**), guanosine (**13**), and guanosine 5′-monophosphate (**14**) for structural



consistency and in view of their biochemical importance.[20] This gave a maximum of $14 \times 12 = 168$ quantities that describe the adsorption of these solutes on HA. The number acquired in practice is 111 (Table S1[1,2]).[21]

The data sets [v_i, K_i, (ΔH_i, ΔS_i)] and derived quantities for adsorption of solutes **1**−**14** in sequential steps A, B, and C will be used to develop an adsorption model for compost-derived HA. We will emphasize the use of average and relative quantities because (a) HAs are heterogeneous materials[9−12] and (b) average and relative quantities help to subclassify the adsorption data for different solutes.

Site Capacities for Sequential Solute Adsorption

Table 1 collects capacities v_i for adsorption of each solute in steps A, B, and C. The first entry is the measured value (for example, $v_A = 0.13$ mmol/g HA for solute **1**) at 25.0 °C. The second is the average value (for example, $\langle v_A\rangle = 0.07$ mmol/g HA for solute **1** over the experimental temperature range 10.0−40.0 °C). The third is the total site capacity $\sum\langle v_i\rangle$, which is the

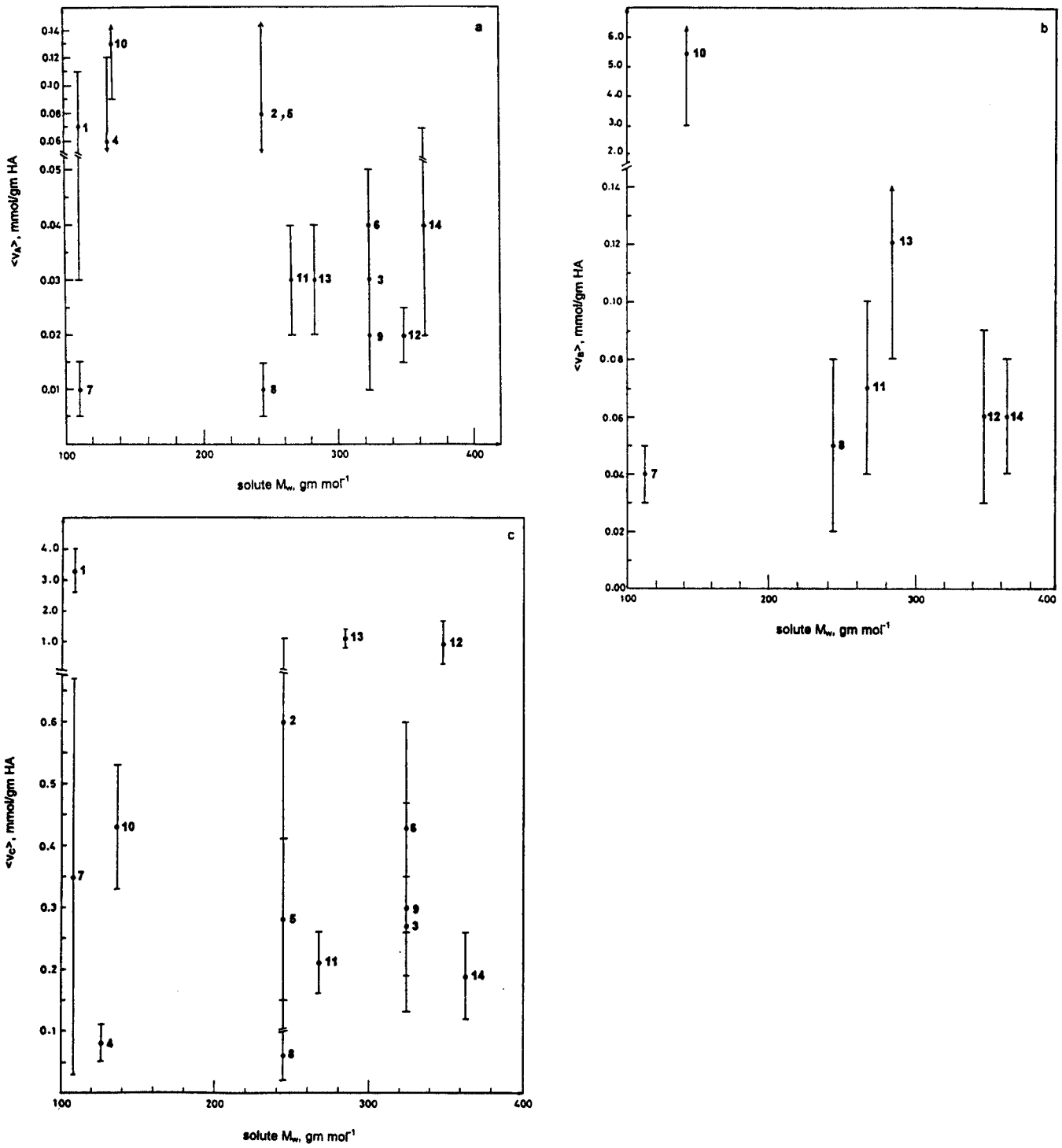


Figure 1. Plots of average site capacity $\langle v_i \rangle$ vs solute molecular weight for nucleic acid constituent adsorption on compost-derived humic acid in (a) step A; (b) step B; (c) step C. Bars indicate average data errors (Table S1).[1,2,21]

sum of the previous three entries in each solute row. For example, $\sum\langle v_i \rangle = 3.37$ mmol/g HA for solute **1**. The data are from Table S1,[21] which indicates data precision. References 1 and 2 describe the measurements and Langmuir analysis of the data.

Inspection of Tables S1 and 1 indicates reasonable agreement between measured v_i at 25.0 °C and the average $\langle v_i \rangle$ over the temperature range 10.0−40.0 °C for adsorption of a given solute in each step. This is encouraging given that v_i are obtained from the intercepts of a plot of $1/A$ vs $1/c$, eq 1, where A is the amount of solute adsorbed per g of HA and c is the solute concentration at equilibrium. A plot of eq 1 results in distinct linear segments for each isotherm (see, for example, Figure 4 of ref 2). The segment intercepts $1/v_i$ are much less precise than the slopes. For this reason the average values $\langle v_i \rangle$ are better for analysis than v_i at one temperature.

$$1/A = 1/v_i + 1/Kv_ic \tag{1}$$

We concluded within the precision limits of the data that v_i for a particular adsorption step and solute is temperature-independent.[1,2] This justifies Langmuir analysis of the data.

Table 1 shows that $\langle v_C \rangle > \langle v_B \rangle > \langle v_A \rangle$ except for the underlined entry. The $\langle v_i \rangle$ data in Table 1 are plotted as a function of solute molecular weight in Figure 1, which indicates the following within the limits of data precision.

Step A. Site capacity $\langle v_A \rangle$ is small and may increase with increasing solute molecular weight from 0.01 mmol/g HA for

uracil (**7**) to 0.04 mmol/g HA for guanosine 5′-monophosphate (**14**), Figure 1a. Another interpretation within the data precision limits is that $\langle v_A \rangle$ is independent of solute molecular weight for cytidine 5′-monophosphate (**3**), thymidine 5′-monophosphate (**6**), uridine 5′-monophosphate (**9**), adenosine (**11**), adenosine 5′-monophosphate (**12**), guanosine (**13**), and guanosine 5′-monophosphate (**14**) and that $\langle v_A \rangle$ for uracil (**7**) and uridine (**8**) are unexpectedly low. Parameter $\langle v_A \rangle$ is 0.026 ± 0.008 mmol/g HA (i.e. ±30%) if the data for **7** and **8** are included, and $\langle v_A \rangle$ = 0.030 ± 0.005 mmol/g HA (i.e. ±17%) if they are not. Precision is better if the data for **7** and **8** are not included.

Thus, $\langle v_A \rangle$ for uracil (**7**) and uridine (**8**) are anomalously low. With either interpretation, $\langle v_A \rangle$ for cytosine (**1**), cytidine (**2**), thymine (**4**), thymidine (**5**), and adenine (**10**) are much greater than expected, and $\langle v_A \rangle$ for adenine is especially large. Excluding the adenine data, $\langle v_A \rangle$ for solutes **1**, **2**, **4**, and **5** is 0.070 ± 0.013 mmol/g HA (i.e. ±18%).

We conclude that seven of the fourteen solutes have $\langle v_A \rangle$ = 0.030 ± 0.005 mmol/g HA, two (uracil and uridine) have $\langle v_A \rangle$ = 0.01 ± 0.005 mmol/g HA, and four (cytosine, cytidine, thymine, and thymidine) have $\langle v_A \rangle$ = 0.070 ± 0.013 mmol/g HA. $\langle v_A \rangle$ for adenine adsorption is far larger than that for any other solute (Table 1).

Step B. Adsorption in step B was only detected for seven of the fourteen solutes.[1,2] Figure 1b illustrates that $\langle v_B \rangle$ for uracil (**7**), uridine (**8**), adenosine (**11**), adenosine 5′-monophosphate (**12**), and guanosine 5′-monophosphate (**14**) is 0.054 ± 0.009 mmol/g HA (i.e. ±17%) and is independent of solute molecular weight within the data precision. However, $\langle v_B \rangle$ for guanosine (**13**) is 0.12 ± 0.04 mmol/g HA, and $\langle v_B \rangle$ for adenine is 45−100 times larger than for the other solutes (Table 1).

Step C. Three kinds of behavior are apparent in adsorption step C (Figure 1c).

1. The $\langle v_C \rangle$ for solutes **2**, **3**, **5**, **6**, **7**, **9**, **11**, and **14** range from 0.17 to 0.6 mmol/g HA, with an average $\langle v_C \rangle$ of 0.32 ± 0.11 mmol/g HA (i.e. ±34%) if the cytidine (**2**) data are included and 0.28 ± 0.07 mmol/g HA (i.e. ±25%) if they are not. In either case, $\langle v_C \rangle$ is statistically independent of solute molecular weight based on the known data precision.

2. The $\langle v_C \rangle$ for adenosine 5′-monophosphate (solute **12**, 0.95 mmol/g HA), guanosine (**13**, 1.1 mmol/g HA), and cytosine (**1**, 3.3 mmol/g HA) are statistically too large to average with the above data and they cover a wide range.

3. The $\langle v_C \rangle$ data for thymine (**4**, 0.08 mmol/g HA) and uridine (**8**, 0.06 mmol/g HA) are too small to fit with the above data. They average 0.07 ± 0.01 mmol/g HA (i.e. ±15%) and do not appear to depend on solute molecular weight.

In summary, $\langle v_i \rangle$ in a given adsorption step averages well for the following solutes with different solute molecular weight: step A, **3**, **6**, **9**, **11**, **12**, **13**, **14**; step B, **7**, **8**, **11**, **12**, **14**; step C, **2**, **3**, **5**, **6**, **7**, **9**, **11**, **14**. This suggests only one molecular area of attachment to HA for a solute family like uracil (**7**), uridine (**8**), and uridine 5′-monophosphate (**9**).[1,2]

Comparison with Literature Data

There is a shortage of literature data to include in this comparison and analysis. Structures of solutes that have been studied in the detail needed to give Langmuir parameters v and K are shown in Figure 2. The data are in Table S2.[21]

A study of adsorption of the moderately polar herbicide acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid, **15**, pK_a 3.5) was made with four solute concentrations in the range 25−100 mM, with 20 mg humic acid aliquots in each experiment and at pH 3.2−7.0 (adjusted with aqueous NaOH) and 25 °C.[22] Amounts of **15** adsorbed were measured after 20 h, after which time the supernatant was found to contain

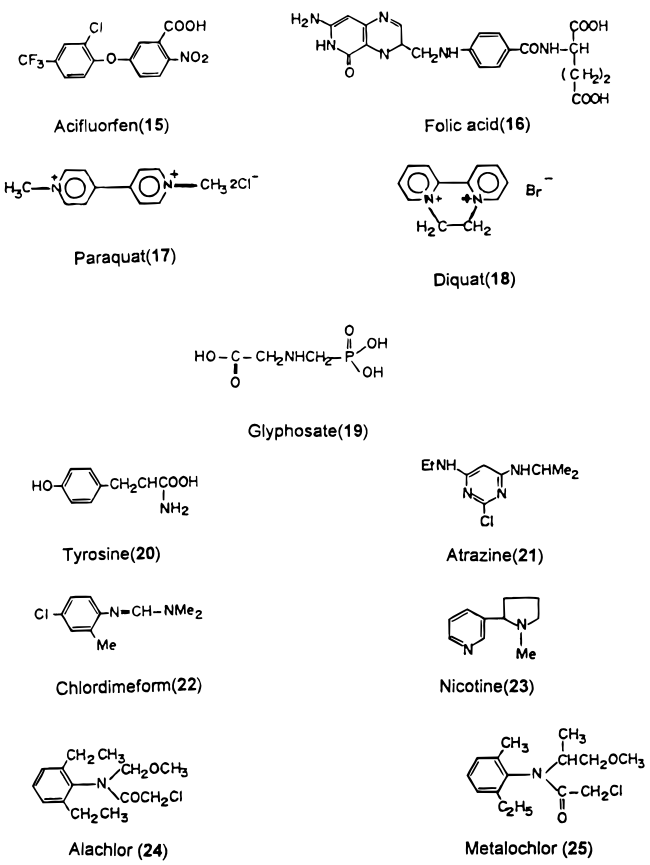


Figure 2. Solutes that adsorb on humic acids (see Table S2).[21]

solubilized HA and equilibrium molar concentrations [**15**]. The solid residue was concluded to be a **15**−HA complex. Langmuir adsorption parameters for this system in Table S2[21] show that the measured site capacity v has a maximum value at pH 3.2.[22]

Solute folic acid (**16**) adsorbs reversibly on a model HA.[23] The Langmuir parameters in Table S2 indicate that the measured site capacity increases by a factor 2 on increasing the temperature from 10 to 35 °C. The two widely used pesticides paraquat (1,1-dimethyl-4,4′-bipyridinium dichloride, **17**) and diquat (1,1-diethylene-2,2′-bipyridinium dibromide, **18**) reversibly adsorb on HA.[24] In these experiments, 10 mg of HA was shaken in water (15 mL) for 1 h and then the solid was treated with 0.05 M **17** or **18** and left to stand at 25 °C for 24 h. The treated solids were examined by IR, which suggested a charge-transfer interaction. The measured site capacities are 0.10 and 0.16 mmol/g HA, respectively (Table S2).[21,24]

An iron-loaded HA sample was found to adsorb the aqueous herbicide glyphosate (**19**) reversibly with a measured site capacity 0.059 mmol/g HA (Table S2).[25] (*S*)-Triazines are very strongly adsorbed by HA at 18 °C in what appears from IR analysis to be salt formation with −COOH as the proton donor.[26] The apparent site capacity of a synthetic HA for tyrosine (**20**), 0.070 mmol/g HA (Table S2), comes from an adsorption study at pH 10 and product analysis by gel filtration.[27]

A careful study of adsorption of atrazine (**21**, 3−40 mM solute, 0.2 g of HA in methanol) was made with an untreated HA and with methylated, calcium-saturated, and diazomethane-treated HA samples. The untreated HA has the largest measured v, and v for this system increases with increasing temperature from 3 to 35 °C (Table S2).[28] Adsorption of the aqueous pesticide chlordimeform (**22**, Figure 2) at 1−50 mM with 50 mg aliquots of untreated, calcium-saturated, iron(III)-saturated, acetylated, methylated, and heated (250 °C) HA after 24 h was studied at 25 °C. Langmuir behavior at a single site was reported (Table S2).[29]

Aqueous (−)nicotine (**23**) adsorbs strongly on HA.[30] A study with [**23**] up to 12.0 mM revealed that HA dissolves at the higher solute concentrations in an important HA "capping" reaction to be discussed below. Useful comparative data for Langmuir adsorption of the aqueous herbicides alachlor (**24**) and metalochlor (**25**) on two forms of activated charcoal (Gro-safe and Norit) are available.[31] Studies with aqueous solutes **24** and **25** at 0−40 mM in the temperature range 5−35 °C indicate temperature-independent site capacities υ that resemble our $\sum\langle\upsilon_i\rangle$. Norit charcoal has higher capacity than Gro-safe for both solutes (Table S2).[31]

The literature data are compared with our average $\langle\upsilon_i\rangle$ for adsorption steps A, B, and C in Table S2.[21] The value for solute **15** at pH 3.2 is similar to our $\langle\upsilon_A\rangle$ but drops sharply with increasing pH.[22] The site capacity υ for **16** at 35 °C also resembles $\langle\upsilon_A\rangle$ for nucleic acid constituents but it decreases with decreasing temperature.[23] The site capacity for **17**[24] approximates the sum $\langle\upsilon_A\rangle + \langle\upsilon_B\rangle$ from our work[1,2] and that for **18**[24] could be $\langle\upsilon_A\rangle + \langle\upsilon_B\rangle + \langle\upsilon_C\rangle$ with lower $\langle\upsilon_C\rangle$ than our value (this also may be the case for atrazine (**21**)[28]). Reducing the temperature, loading the HA with calcium, or treating it with diazomethane with **21** as solute reduces υ to our $\langle\upsilon_A\rangle$, while methylating the HA gives υ approximating $\langle\upsilon_A\rangle + \langle\upsilon_B\rangle$.[28]

Perhaps the most striking feature of Table S2[21] is similar site capacities to ours except for much smaller υ_i for solute **15** at pH 5.0 and 7.0, which is at the upper end of our pH range (pH 6.8).[1,2] Also notable is that two activated charcoal forms have about the same single-site capacities for two herbicides, **24** and **25**, as the total capacity $\sum\langle\upsilon_i\rangle$ of our HA for nucleic acid constituents that have average absorptive properties.

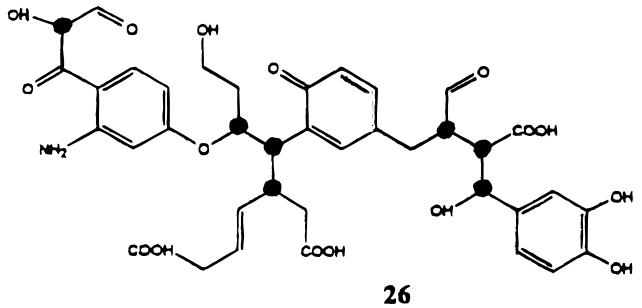
Site Capacity Applications

Our view of HA primary structures is that they consist of one or more building blocks that may be covalently linked in a limited number of ways.[11] Consideration of the stoichiometric origin of adsorption site capacities leads to eq 2. Here, S is the

$$S\upsilon = S\chi_f\upsilon_{max} = 1000S/\langle M_w\rangle \qquad (2)$$

stoichiometry of the interaction of the solute with an adsorbent site, χ_f is the mole fraction of accessible adsorption sites, υ_{max} is the site capacity of the adsorbent with the lowest molar mass, and $\langle M_w\rangle$ is the average adsorbent molar mass.[32]

Proposed HA average building block **26**[11a] has 13 functional groups and a molecular weight of 765 Da. If one mole of a solute reacts with each of the 13 functional groups, then 13 mol of solute react and the structure is "capped." Therefore,



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υ_{max} for this structure is 13,000 mmol solute/765 g **26** = 17.0 mmol/g HA, which is in excellent agreement with experiment: the site capacities υ_A, υ_B and υ_C for adsorption of (−)nicotine (**23**) on a river-sediment-derived HA in Table S2[21] add up to 17.0 mmol/g HA.[30] Average HA building block **26** is consistent with complete disruption of aggregated HA molecules into soluble HA building blocks by reaction with a solute like (−)-nicotine.[30] Parameter υ_{max} is independent of whatever solute completely caps structure **26**.[34] The caps prevent interactions of building block functional groups that re-form larger HA molecules or aggregates.

TABLE 2: Summary of Langmuir Parameters for Adsorption[a]

step	average value	too small	too large
	Capacities (mmol/g HA)		
A	0.030 ± 0.005	uracil uridine	adenine cytosine cytidine thymine thymidine
B	0.054 ± 0.009		adenine guanosine
C	0.28 ± 0.07	thymine uridine	cytosine guanosine adenosine-P
	Equilibrium Constants (M^{-1})		
A	$(2.1 \pm 0.9) \times 10^4$	Thymidine-P	guanosine guanosine-P uracil uridine-P
B	$(3.6 \pm 1.1) \times 10^3$	adenine	adenosine-P guanosine
C	$(1.5 \pm 0.2) \times 10^3$	guanosine cytosine uridine	adenine cytidine cytidine-P uridine-P

[a] Entries that appear more than once in a column are underlined.

If $\langle\upsilon_i\rangle$ for a series of solutes adsorbed on HA in step i = A, B, or C clearly fall into subsets where $\langle v_i\rangle$ differ by a factor of 2, then one solute subset occupies twice as many of the same sites A, B, or C as the other. If there are no clear $\langle\upsilon_i\rangle$ value ratios (as for the data in Table 1), then adsorption of a given solute at a given site must cause changes on the adsorbent surface or its state of aggregation[12] or its molecular weight. Unexpectedly large values of $\langle\upsilon_i\rangle$ account for the appearance of S-curves in adsorption isotherms.[35] They are a strong predictor of adsorbent disaggregation or chemical fragmentation, as occurs when solutes like adenine[2] and (−)nicotine (**23**)[30] adsorb on humic acids.

Adsorption Sites

We propose the idea that specific adsorption of nucleic acid constituents on compost-derived HA in step A, B or C involves respective HA functional groups A, B, and C with different chemical identities. Evidence from the temperature dependence of equilibrium constants that supports this view will be discussed in a later section.

Equation 2 with known or calculated υ_{max} can be used to estimate the mole fraction of accessible adsorption sites in a solid adsorbent such as a humic acid or a charcoal. For example, $\langle\upsilon_A\rangle$ for adsorption of seven nucleic acid constituent solutes taken to be average in step A is $\langle\upsilon_A\rangle = 0.030$ mmol/g HA (Table 2). Substitution of this value with $S = 1$ and $\upsilon_{max} = 17.0$ mmol/g HA in eq 2 gives $\chi_f = 0.030/17.0 = 1.76 \times 10^{-3}$. Thus, only 0.18% of the sites of a building block like **26** are available for adsorption of the average solute in step A. From Table 1, the corresponding percentages for adsorption of the average solutes in steps B and C are 0.32% and 1.6%, respectively. Thus, only 2.1% of the functional groups in proposed building block **26** are available for adsorption of average nucleic acid constituent solutes on compost-derived HA.

Suppose an HA particle has 1 mol of site A that can react with 1 mol of a solute (that is, $S = 1$ in eq 2). The average $\langle\upsilon_A\rangle$ is 0.030 mmol/g HA (Table 2), so an HA molecule with 1 mol of sites A weighs 33.3 kDa from eq 2 with $\langle M_w\rangle = 1000/$

TABLE 3: Number and Relative Number of Moles of Sites for Nonaverage Nucleic Acid Constituent Solutes Adsorbing on an Average 30.9 kDa HA Particle[a]

solute	n_A	rel n_A	n_B	rel n_B	n_C	rel n_C
adenine (**10**)	4.0	13	170	110	13	7
adenosine-P (**12**)	0.6	2	2	1.2	29	16
guanosine (**13**)	0.93	3	4	2.4	40	22
cytosine (**1**)	2.2	7	*b*	*b*	100	55
cytidine (**2**)	2.5	8	*b*	*b*	7.4	4[c]
					28	15[d]
average[e]	0.93	3	1.7	1.1	8.7	4.7
thymine (**4**)	1. 9	6	*b*	*b*	2.5	1.3
uridine (**8**)	0.31	1.0	1.6	1.0	1.9	1.0

[a] See text for computation method and Table 2 for summary of nonaverage solute behavior. Rel n_i values are independent of sorbent M_w since $n_i = \langle M_w \rangle \langle v_i \rangle$ and $\langle M_w \rangle$ is fixed at 30.9 kDa in the calculations of n_i shown. [b] Adsorption step B is not detectable with these solutes (see Table 1). [c] From $\langle v_C \rangle$ estimate at temperatures at or below 25.0 °C (ref 1). [d] From $\langle v_C \rangle$ estimate at higher temperatures (ref 1). [e] Behavior of average solutes on average 30.9 kDa HA particle (see text).

$\langle v_A \rangle$. Calibrated gel filtration chromatography of the HA of this study (Sephadex G-50, pH 9.0 eluent at 25 °C) gave four fractions (M_w = 12.1 kDa (39%), 17.3 kDa (10%), 24.8 kDa (21%), and 64.0 kDa (30%)) corresponding to average $\langle M_w \rangle$ = 30.9 kDa, which is in good agreement with $\langle M_w \rangle$ = 33.3 kDa from the above assumptions. The fractions eluted from the column are taken to be different or differently aggregated HA molecules.[12]

The average HA particle would contain 30900/765 = 40.3 building blocks **26** and therefore 40.3 × 13 = 525 mol of potential functional groups. The number actually available to the average solute of this study is 0.021 × 525 = 11.

Moles and Relative Numbers of HA Adsorption Sites

In an earlier section we concluded that $\langle v_A \rangle$ = 0.030, $\langle v_B \rangle$ = 0.054, and $\langle v_C \rangle$ = 0.28 mmol/g HA are average site capacities for adsorption in steps A, B, and C, respectively (Table 2). The sum of these values is the average total site capacity $\Sigma\langle v_i \rangle$ = 0.36 ± 0.07 mmol/g HA, whose precision is set by the precision of $\langle v_C \rangle$. Thus, any value of $\Sigma\langle v_i \rangle$ in the range 0.29−0.43 mmol/g HA indicates average solute total adsorption behavior.

Inspection of the last column of Table 1 indicates the following: (1) $\Sigma\langle v_i \rangle$ for solutes cytosine (**1**), cytidine (**2**), adenine (**10**), adenosine 5′-monophosphate (**12**), and guanosine (**13**) are well above average; and (2) $\Sigma\langle v_i \rangle$ for solutes thymine (**4**) and uridine (**8**) are significantly lower than average.

An average HA particle with $\langle M_w \rangle$ = 30.9 kDa would contain 30900/33000 = 0.93 mol of site A. From the ratios $\langle v_B \rangle / \langle v_A \rangle$ and $\langle v_C \rangle / \langle v_A \rangle$ we can calculate that the average 30.9 kDa HA particle has 0.93 mols of site A, 1.7 mol of site B, and 8.7 mol of site C. Their ratio is not the same as the ratios of the functional groups in proposed average HA building block **26** because of the way the building blocks are joined together (see below).

To calculate the moles of A sites used by a nonaverage solute like adenosine 5′-monophosphate, whose $\Sigma\langle v_i \rangle$ is higher than average (Table 1, last column), we multiply $v_A / \langle v_A \rangle$ by 0.93, where v_A is the value for that solute at 25.0 °C in Table 1. This gives n_A = 0.6 mol of A sites for adenosine 5′-monophosphate. Similar use of $v_B / \langle v_B \rangle$ and $v_C / \langle v_C \rangle$ with the factors 1.7 and 8.7 for average solutes gives n_A = 0.6 mol of A sites, n_B = 1.9 mol of B sites, and n_C = 29 mol of C sites available for adsorption of adenosine 5′-monophosphate (**12**) on the 30.9 kDa HA particle (Table 3).

The same calculations for nonaverage solutes cytosine (**1**), cytidine (**2**), thymine (**4**), uridine (**8**), adenine (**10**), and guanosine (**13**) give the data in Table 3. We see that uridine adsorbs on the smallest number of moles of A (0.31), B (1.6), and C (1.9) sites of the 30.9 kDa HA particle. We adopt uridine (**8**) as a standard solute, and express all the other n_A, n_B, and n_C as relative (for example, rel n_A) to that for uridine in Table 3. These numbers indicate that the solutes above uridine in Table 3 have more HA sites available to them. For example, adenine (**10**) has 13 times more A sites, 110 times more B sites, and 7 times more C sites available for its adsorption on HA than does uridine. These sites are "freed up" from interactions with other HA components by the primary act of adsorbing, say, cytosine (**1**) on an exposed A site (we see that six more A sites are freed up as a result of adsorption of the first cytosine molecule, Table 3).

Excluding adenine, which has an exceptional ability to access additional A (12) and B (110) sites when it is adsorbed, the average number of moles of new A, B**,** and C sites accessed is 3.6, 0.6, and 16, respectively, for the solutes in Table 3.

A major message of Table 3 is that except for the dramatic behavior of adenine it is much harder to access additional B sites than it is to access more A and C sites as a result of primary solute adsorption. This is why detection of adsorption on site B is not always possible.[1] A given solute can more easily find more C sites than B sites, and so adsorption at site C can overshadow and obscure adsorption at B.

We will use the data in Table 3 again after considering the adsorption equilibrium constants.

Adsorption Equilibrium Constants

The equilibrium constants at 25.0 °C for adsorption in steps A, B**,** and C (Table S1)[21] can be ranked and compared with literature data. The numbering scheme is cytosine (**1**), cytidine (**2**), cytidine 5′-monophosphate (**3**), thymine (**4**), thymidine (**5**), thymidine 5′-monophosphate (**6**), uracil (**7**), uridine (**8**), uridine 5′-monophosphate (**9**), adenine (**10**), adenosine (**11**), adenosine 5′-monophosphate (**12**), guanosine (**13**), and guanosine 5′-monophosphate (**14**). Equilibrium constants K_i increase as follows:[2] step A, **6** < **1** < **2** < **3** < **11** ≈ **4** ≈ **5** < **8** < **12** < **10** < **14** < **13** < **9** < **7**; step B, **10** < **8** ≈ **14** < **7** < **11** < **12** < **13**; step C, **12** < **13** ≈ **1** ≈ **8** < **6** < **7** < **11** ≈ **14** ≈ **5** < **10** < **4** < **2** ≈ **3** < **9**.

The above sequences clearly show that HA sites A, B, and C have different selectivity for nucleic acid constituents. These sequences are taken to be different because sites A, B**,** and C have different chemical identities.[11,34] The above sequences do not relate in any simple way to the elution orders for chromatography of nucleic acid constituents on various stationary phases[36] because chromatographic retention depends on a number of other factors besides stationary phase adsorption.[36]

Comparison of Adsorption Equilibrium Constants with Literature Data

Tables S1 and S2[21] allow comparison of our average K_A, K_B, and K_C (M^{-1}) at 25.0 °C with those for sorption of different solutes on different HA and charcoal systems. Our equilibrium constants most closely resemble those for solutes **15** and **16** (Figure 2). They are much larger than those for solutes **19** and **21** and an order of magnitude smaller than those for adsorption of solutes **24** and **25** on activated charcoals. However, K_A for solutes guanosine ($K_A = 1.0 \times 10^5$), guanosine 5′-monophosphate ($K_A = 7.6 \times 10^4$), uracil ($K_A = 7.8 \times 10^5$), and uridine 5′-monophosphate ($K_A = 3.6 \times 10^5$, Table S1[21]) resemble the data for adsorption of herbicides alachlor (**24**) and metalochlor (**25**) on activated charcoals[31] in Table S2.[21]

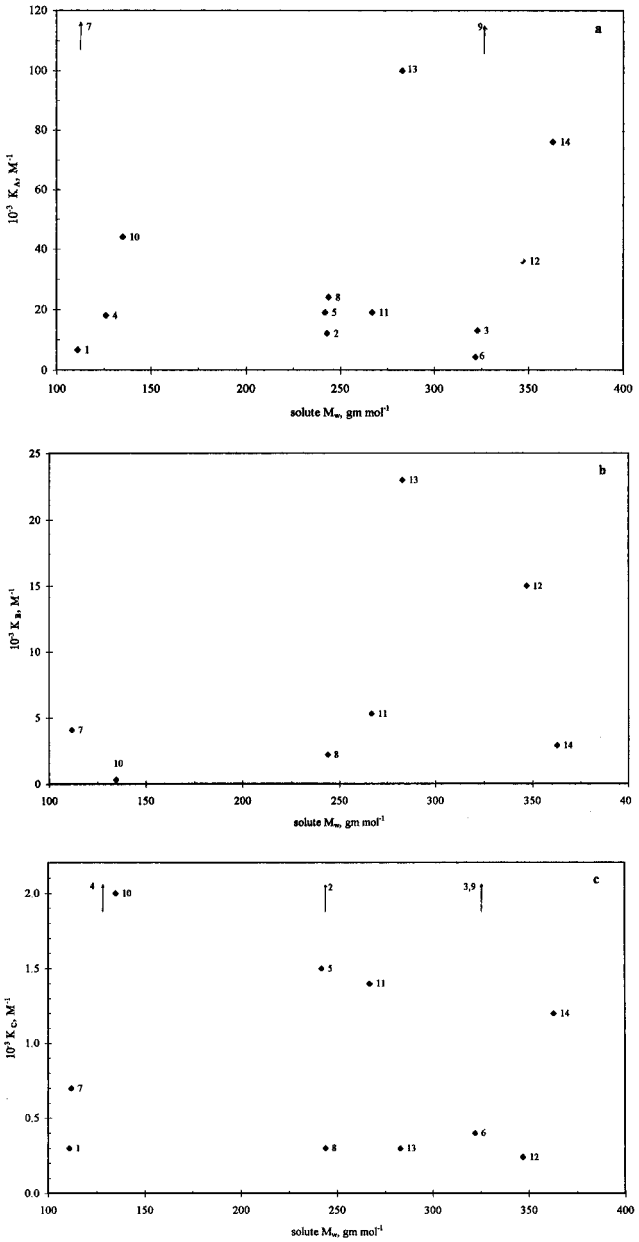

Figure 3. Plots of equilibrium constants K_i at 25.0 °C vs solute molecular weight for nucleic acid constituent adsorption on compost-derived humic acid in (a) step A; (b) step B; (c) step C. See text for solute key.

Independence of Equilibrium Constants K_i on Solute Molecular Weight and Binding Mode

The equilibrium constants (M^{-1}) at 25.0 °C for adsorption of solutes **1**−**14** in steps A, B, and C are plotted as a function of solute molecular weight in Figure 3. The data indicate that $K_C < K_B < K_A$ in general and these other features.

Step A. There appear to be two or perhaps three kinds of equilibrium behavior for adsorption in step A (Figure 3a).

1. The K_A for one group of eight solutes ranges from 1.2×10^4 (cytidine, **2**) to 4.4×10^4 (adenine, **10**), with an average $K_A = (2.3 \pm 0.9) \times 10^4$ (i.e. ±38%) that is independent of solute and of solute molecular weight or charge[37] (for example, compare the data for neutral adenine (**10**) and adenosine (**11**) and monoanionic adenosine 5′-monophosphate (**12**) or for thymine (**4**), thymidine (**5**), and thymidine 5′-monophosphate (**6**) in Figure 3a).

2. The four solutes guanosine (**13**, $K_A = 1.0 \times 10^5$), guanosine 5′-monophosphate (**14**, 7.6×10^4), uracil (**7**, 7.8×10^5) and uridine 5′-monophosphate (**9**, 3.6×10^5) have K_A values much larger than and well outside the above range.

3. Cytosine (**1**) has a relatively small K_A (6.7×10^3) at 25.0 °C. If this value is included, the average K_A for nine of the solutes is $(2.1 \pm 0.9) \times 10^4$ (i.e. ±43%). The K_A for thymidine 5′-monophosphate (**6**, 4.4×10^3) is well below this range.

So within the precision limits of the data, K_A for eight of the fourteen solutes are independent of solute and of solute molecular weight and charge. Four other solutes (**7**, **9**, **13**, and **14**) have much larger K_A, and K_A for solutes **1** and **6** seem low (Figure 3a).

Step B. Five of the seven solutes for which adsorption step B is detected (Tables 1 and S1[21]) have K_B ranging from 2.2×10^3 (uridine (**8**)) to 5.3×10^3 (adenosine, (**11**)), with an average $K_B = (3.6 \pm 1.1) \times 10^3$ (i.e. ±30%) that is independent of solute molecular weight (Figure 3b). The K_B for adenine (**10**, 3.3×10^2) is well below this range, and K_B for adenosine 5′-monophosphate (**12**) and guanosine (**13**) interpolated from the data in Figure 2 of ref 2 are well above it.

Step C. There appear to be three types of behavior for adsorption at HA site C (Figure 3c).

1. Six of the solutes have $K_C = (3.7 \pm 1.1) \times 10^2$ (i.e. ±29%) that are independent of solute molecular weight and charge.

2. Another four solutes have $K_C = (1.5 \pm 0.2) \times 10^3$ (i.e. ±13%) that also are independent of solute molecular weight and charge (Figure 3c). We take this set to be the average K_C in Table 2 (see below).

3. Three solutes, cytidine (**2**), cytidine 5′-monophosphate (**3**), and adenine (**10**), have far larger values $K_C = (5.7 \pm 1.1) \times 10^3$ (i.e. ± 19%). The K_C of uridine 5′-monophosphate (**9**) is well above the range.

Two major conclusions to this point are that (1) most of the nucleic acid constituent adsorption processes occur with temperature-independent site capacities that also are independent of solute molecular weight and charge; and (2) equilibrium constants for adsorption in a given adsorption step can be grouped depending on the solute and also are independent of solute molecular weight and charge. This supports the idea that the solutes of this study attach themselves to HA through their nucleobase units.[1,2] Our conclusions are summarized in Table 2. Entries that appear more than once in an exception column are underlined.

Relationship between Equilibrium Constants and Site Capacities

Figure 4 explores the relationships between relative K_A, relative K_B, and relative K_C and relative n_A, n_B, and n_C, respectively, with uridine (**8**) (rel K_A = rel n_A, etc. = 1) as the standard solute. The rel K_i values are calculated from the data in Table S1,[21] and relative n_i estimates are from Table 3. The points of focus are the nonaverage solute behavior in Table 2 and separate consideration of steps A, B, and C.

Step A. Figure 4a shows that adsorption of **12** and **13** in step A has different characteristics compared to that of an average solute and solutes **1**, **2**, **4**, and **8**. For **12** and **13**, availability of more A sites requires increasingly larger relative K_A, while smaller rel K_A results in increased site A availability in the sequence **8**, average solute, **4**, **1**, and **2**. These differences indicate that it is the chemical character of a solute, rather than the adsorption equilibrium constant itself, that determines its ability to "free up" new A sites on HA. Solutes **12** and **13** clearly have different properties that influence their interaction with site A. Solute **10** (adenine) is in a class by itself.

Step B. Solute **12** behaves like an average solute in its interaction with site B (Figure 4b). We are not sure why solute

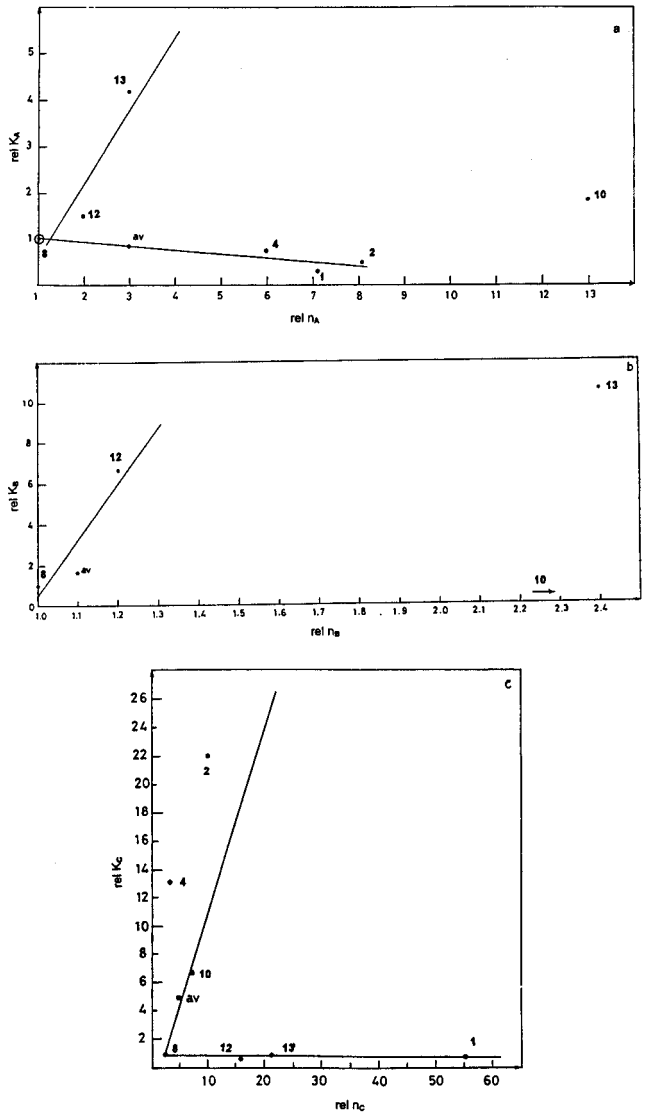

Figure 4. Plots of rel K_i vs rel n_i for nucleic acid constituent adsorption on compost-derived humic acid in (a) step A; (b) step B; (c) step C. The data are from Tables 2 and S1.[21] See text for solute key.

13 has larger than expected ability to free up B sites. Once again, solute adenine (**10**) is in a class by itself: it has enormous relative ability to access B sites.

Step C. On the other hand, adenine is average in its reaction with site C, but now **2** and **4** seem to have difficulty accessing C sites (Figure 4c). Solutes **12**, **13**, and **1** behave like solutes on the lower line of Figure 4a in their interactions with site C.

A Model for Adsorption of Nucleic Acid Constituents on Compost-Derived HA

The best evidence that adsorption steps A, B, and C involve chemically different respective groups A, B, and C is that a plot of log K_i vs $1/T$ for each solute in each detectable step is linear. Such linearity is expected for interaction of fixed reactants (for example, reaction of thymine with a carboxylic acid group in step A). From this it is reasonable to conclude that all the solutes react with the same specific group (for example, a carboxylic acid group) in one of the steps but with different HA functional groups in the other steps. The capacity of HA for adsorption of a particular solute in a step depends on the solute's ability to access the functional group involved in that step.

The data and analysis in this paper suggest the following.

1. The adsorption of a nucleic acid constituent in steps A, B, and C occurs by attachment of its nucleobase. We envision that the nucleosides and nucleotides are situated in the aqueous phase with their pentose and phosphoric acid components away from the HA surface.

2. Depending on the solute, the primary adsorption act can free up additional sites near the first site of attachment.[38] Some interactions A···X,Y that are broken may be physical (for example, hydrogen-bonding interactions)[11b,12] while others may be chemical (for example, hydrolysis of an ester to give an alcohol functional group and a carboxylic acid group, with the carboxylic acid being site A). The latter process "creates" a sorbent site that otherwise would not be available to the solute. Choice of uridine (**8**) as a standard amounts to neglecting the breaking of (A, B, C)···X,Y interactions as a result of primary adsorption of this solute. In other words, uridine adsorbs only on sites A, B, or C that are not involved in (A, B, C)···X,Y interactions.

Figure 5 suggests how solute adsorption could occur. We imagine that surface site A has nearby groups A that are involved in interactions with different HA groups X, Y. Access to such A sites requires the solute to disrupt interactions A···X,Y.

Such interactions could include bonds that link building blocks like **26** to form humic acid polymer molecules. One of the characteristics of the known functional groups of HA[9−11] is that they condense with each other to form amides, carbamides, esters and phenylesters, eqs 3−6, where the first reactant is provided by one building block and the second by another.

$$-NH_3^+ + HOOC- \leftrightarrow -^+NH_2-C(O)- + H_2O \quad K_{am} \qquad (3)$$

$$-CH(O) + {}^+H_3N- \leftrightarrow -CH{=}NH^+- + H_2O \quad K_{cm} \qquad (4)$$

$$-COH + HOOC- \leftrightarrow -C-O-C(O)- + H_2O \quad K_{es} \qquad (5)$$

$$-PhOH + HOOC- \leftrightarrow -Ph-O-C(O)- + H_2O \quad K_{pe} \qquad (6)$$

Some facts about these reactions are helpful in assessing the proposed model.

1. All the reactions are equilibria, all are reversed by reaction with coproduct water, several are known to be essentially thermoneutral,[39,40] and many of the equilibrium constants have been measured in water.[41,42]

2. The reactions are acid and base catalyzed.[43,44] Humic acids are fragmented by aqueous base (albeit slowly at ambient temperatures).[17] Three amines (adenine (**10**),[2] cytosine (**1**),[1] and (−)nicotine (**23**)[30]) readily dissolve HA at pH's where HAs are insoluble.[45] Reactions 3−6 reflect the likely protonation states of HA functional groups under the conditions of our adsorption measurements.[1,2,41,42] There is no evidence for a pH dependence of the measured equilibrium constants.[1,2]

3. A building block like **26** is capable of many condensation linkages.[34] Those that actually occur should depend on the orientations of the functional groups in the building block (that is, the ease with which the reactive functionalities can be brought together).[46,47] Making several linkages between building blocks is like closing a zipper. The equilibrium constant for formation of four linkages through reactions 3−6 is $K_{am}K_{cm}K_{es}K_{pe}$ and could be substantial.[41,42]

An earlier calculation indicated that only 11 of the 525 moles of functional groups in an average 30.9 kDa HA particle made from 40.3 building blocks **26** are available as adsorption sites for an average solute. Thus, 514/2 = 257 reactions like 3−6 could have linked the 40.3 building blocks together in this average HA particle.

Figure 5, step a shows an A site surrounded by A···X,Y interactions. According to Table 3, adsorption of one guanosine

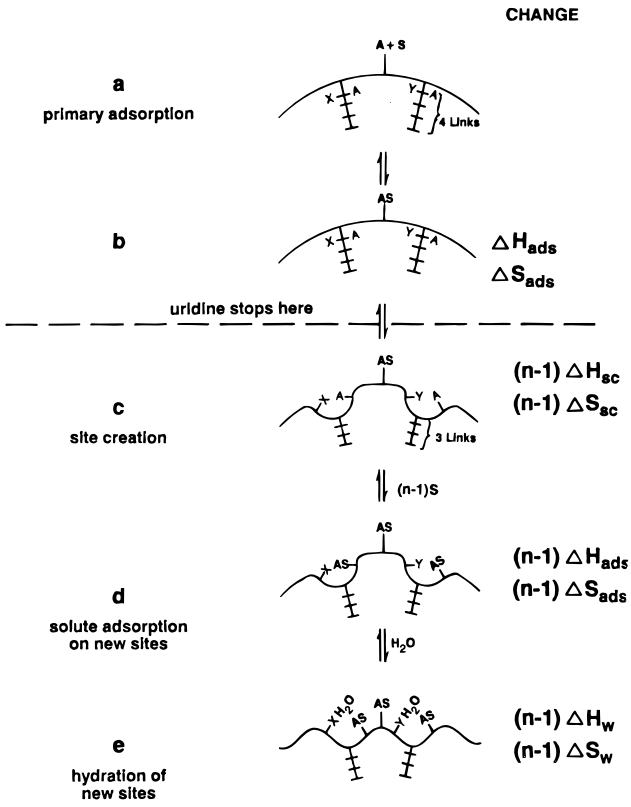

Figure 5. Proposed model for nucleic acid constituent adsorption on compost-derived humic acid.[38,48]

(**13**) solute molecule[37] on site A results in the availability of two more A sites, perhaps through reversal of one reaction or others like 3−6 (Figure 5, step b). Guanosine adsorbs reversibly on these two additional A sites (Table 3), as proposed in step d of Figure 5.[38] We propose that the functional groups X, Y etc. (not A) released by disruption of two A···X,Y interactions on separate zippers are hydrated by water in step e of Figure 5.[38,48] The same sequence of events (but involving different functional groups B and C in B···X,Y and C···X,Y interactions) is proposed to account for solute adsorption in steps B and C, respectively.

We will now discuss this model with the aid of the adsorption thermodynamics.

Adsorption Thermodynamics

We found that adsorption of nucleic acid constituents on compost-derived HA can be endothermic, thermoneutral, or exothermic, depending on the adsorption step and solute.[1,2] In these systems, endothermic adsorption always is accompanied by a positive entropy change, and exothermic adsorption always results in an entropy decrease of the system (Table S1).[21]

One of the best ways to recognize any relationship between heat and entropy changes is to plot ΔH vs ΔS. Some comments on this approach are necessary before we present the results.

Consider eqs 7 and 8 (the symbols have their usual significance).[43,44]

$$\Delta G = \Delta H - T\Delta S \tag{7}$$

$$\Delta G^\circ = -2.303RT \log K \tag{8}$$

Firstly, the range of measured equilibrium constants (M^{-1}) at 25.0 °C in Table S1[21] is from 0.24×10^3 (for adenosine 5′-monophosphate (**12**) adsorption in step C) to 7.8×10^5 (uracil (**8**) adsorption in step A). This corresponds to $\Delta G^\circ = -3.2$ to -8.1 kcal mol^{-1}, which is much smaller than the range of ΔH values in Table S1. Rearranging eq 7 to give eq 9 shows that ΔH should be a linear function of ΔS because $\Delta G = \Delta G^\circ$ is effectively constant.[49]

$$\Delta H = T\Delta S + \Delta G \tag{9}$$

Suppose we have a series of reactions with different mechanisms or with very dissimilar reactants. Plotting eq 9 for such data should indicate no correlation of ΔH with ΔS or at best segregate the data on lines that are displaced from each other and/or have different slopes. This approach has been used to subclassify sets of kinetic and equilibrium data for wide ranges of oxidation−reduction and metal exchange reactions[50,51] and to draw conclusions about their mechanisms.[49c,50] Application of eq 9 to the data in Table S1[21] results in a remarkable linear correlation (given as Figure S1),[21] which accommodates every (ΔH_i, ΔS_i) data pair in Table S1. The slope of Figure S1 is 297 K (24 °C) and the intercept is -5.2 kcal mol^{-1}, the average ΔG° of the range -3.2 to -8.1 kcal mol^{-1} for adsorption of solutes **1**−**14** in steps A, B, and C (Table S1).[21]

Figure S1 strongly suggests that each of the adsorption steps detected has the same sort of underlying molecular mechanism. This encourages us to explore the adsorption model further. We have to account for the very wide range of adsorption enthalpies and entropies in Figure S1.[21]

Relative Thermodynamic Parameters and the Adsorption Model

We can write eqs 10−13 to express the measured thermodynamic parameters for adsorption at each HA site A, B, or C in terms of the adsorption model in Figure 5. Here, subscripts

$$\Delta H_{obsd} = n\Delta H_{ads} + (n-1)(\Delta H_{sc} + \Delta H_w) \tag{10}$$

$$\Delta H^\circ_{obsd} = \Delta H^\circ_{ads} \tag{11}$$

$$\Delta S_{obsd} = n\Delta S_{ads} + (n-1)(\Delta S_{sc} + \Delta S_w) \tag{12}$$

$$\Delta S^\circ_{obsd} = \Delta S^\circ_{ads} \tag{13}$$

obsd, ads, sc, and w refer to a measured quantity, associated with solute adsorption, associated with site creation, and associated with site hydration, respectively, and n is the number of moles of occupied adsorption sites A, B, or C (Table 3 and Figure 5). The degree sign refers to a standard system. As in calculating the relative numbers of occupied sites and their relationship to equilibrium constants K_i, we adopt uridine (**8**) as the standard solute and assume on the basis of its low site capacities $\langle v_i \rangle$ that its adsorption involves no disruption of interactions (A, B, C)···X,Y.

Dividing eq 10 by eq 11 and eq 12 by eq 13 and rearranging gives eqs 14 and 15, respectively, where a = rel n_i, b = (rel n_i − 1)/rel n_i, $\alpha = \Delta H_{sc} + \Delta H_w$, and $\beta = \Delta S_{sc} + \Delta S_w$. Defined

$$\text{rel } \Delta H_{obsd}/a = \Delta H_{ads}/\Delta H^\circ_{ads} + b(\Delta H_{sc} + \Delta H_w)/\Delta H^\circ_{ads} = \text{rel } \Delta H_{ads} + b\alpha/\Delta H^\circ_{ads} \tag{14}$$

$$\text{rel } \Delta S_{obsd}/a = \Delta S_{ads}/\Delta S^\circ_{ads} + b(\Delta S_{sc} + \Delta S_w)/\Delta S^\circ_{ads} = \text{rel } \Delta S_{ads} + b\beta/\Delta S^\circ_{ads} \tag{15}$$

quantities a and b can be calculated from the information in Table 3 and used for analysis of the thermodynamic parameters for adsorption at each HA site i = A, B, or C.

For example, adsorption of any solute on site A can be examined with a plot of rel $\Delta H_{obsd,A}/a$ vs b, where a = rel n_A and b = (rel n_A − 1)/rel n_A. The result should be a straight line with a unit intercept and a slope of $(\Delta H_{sc} + \Delta H_w)/\Delta H^\circ_{ads,A}$. Similarly, a plot of rel $\Delta S_{obsd,A}/a$ vs b should have a unit

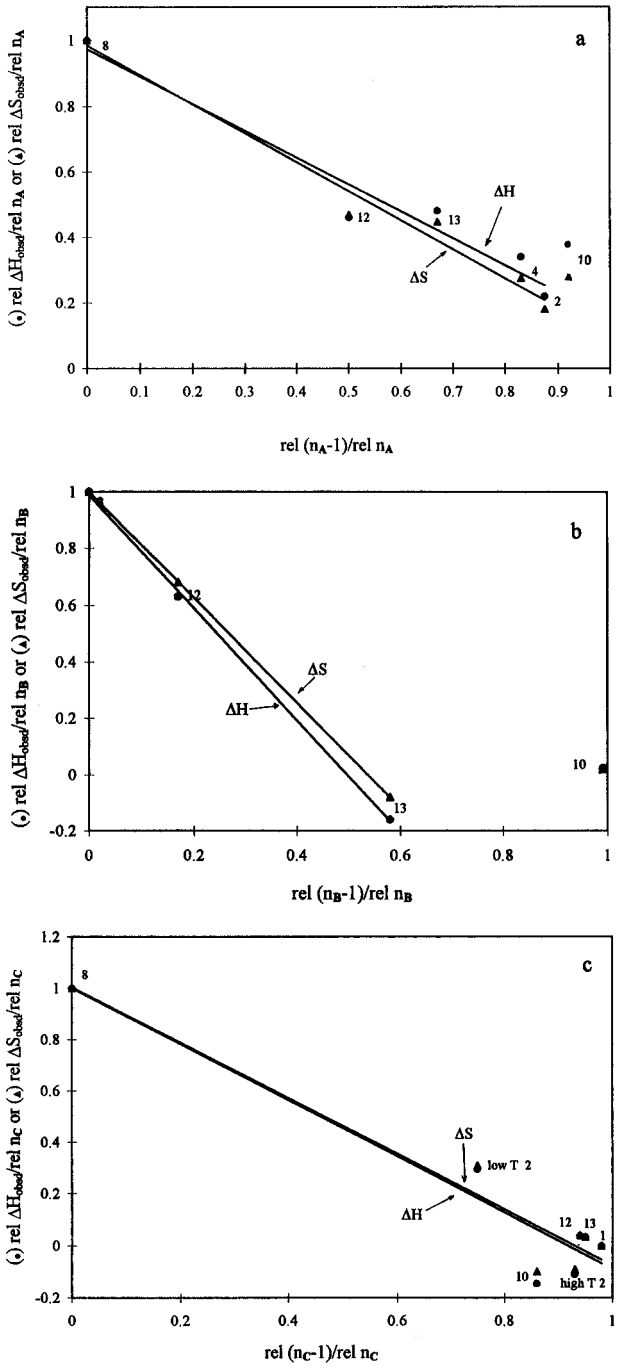

Figure 6. Plots of (●) rel ΔH_{obsd}/rel n_i or (▲) rel ΔS_{obsd}/rel n_i vs rel $(n_i - 1)$/rel n_i for nucleic acid constituent adsorption on compost-derived humic acid at (a) i = A, site A; (b) i = B, site B; (c) i = C, site C. The data are from Tables 3 and S1.[21]

intercept and a slope of $(\Delta S_{sc} + \Delta S_w)/\Delta S°_{ads,A}$. Separate plots can be made for sites A, B, and C.

Plots for solutes that access additional sites on primary adsorption (Figure 6) were made with the data in Tables S1[21] and 3. The results are good straight lines with negative slopes and the expected unit intercepts. Since $\Delta H°_{ads,i}$ and $\Delta S°_{ads,i}$ for adsorption of standard solute uridine (**8**) at each of sites i = A, B, and C are known (Table S1[21]), we can calculate $(\Delta H_{sc} + \Delta H_w)$ and $(\Delta S_{sc} + \Delta S_w)$ by substitution into the plot slopes. The results are collected in Table 4.

Within the precision limits of the data, all the solutes except adenine (**10**) behave as expected. Adenine adsorption at sites A and B involves more sites than expected from its rel $\Delta H_{A,B}$ and rel $\Delta S_{A,B}$ values (Figure 6a,b). We already know that adenine has an exceptional ability to access sites A and especially B, as indicated by its large $\langle \upsilon_A \rangle$ and $\langle \upsilon_B \rangle$ (Table 1).

TABLE 4: Calculated Enthalpy and Entropy Changes at HA Sites A, B, and C

site	$\Delta H°_{ads}$ [a,b]	$\Delta S°_{ads}$ [b,c]	$(\Delta H_{sc} + \Delta H_w)$[a]	$(\Delta S_{sc} + \Delta S_w)$[c]
A	13.1	65	−10.8	−58
B	28.2	111	−56.0	−210
C	33.1	123	−36.1	−130

[a] Units are kcal mol^{-1} (typical error ±2 kcal mol^{-1}). [b] Data for uridine (**8**) standard from Table S1.[21] [c] Units are cal deg^{-1} mol^{-1} at 25 °C (typical error is ±5 cal deg^{-1} mol^{-1}).

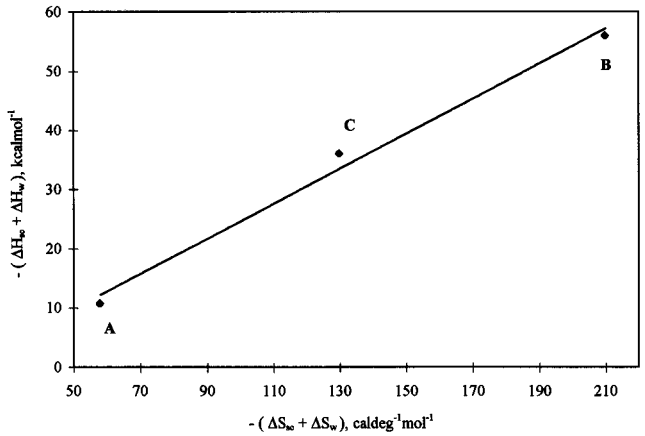

Figure 7. Plot of $(\Delta H_{sc} + \Delta H_w)$ vs $(\Delta S_{sc} + \Delta S_w)$ for nucleic acid constituent adsorption on compost-derived humic acid sites A, B, and C.

The combined steps c and e in Figure 5 are exothermic and result in entropy decreases (Table 4). The most negative $(\Delta H_{sc} + \Delta H_w)$ and $(\Delta S_{sc} + \Delta S_w)$ is for adsorption of "site-creating" solutes at site B. Once sites A, B, and C and their interacting partners X, Y, etc. have been chemically identified in HA, we will be able to dissect $(\Delta H_{sc} + \Delta H_w)$ and $(\Delta S_{sc} + \Delta S_w)$ into their separate components.

Figure 7 shows the correlation of $(\Delta H_{sc} + \Delta H_w)$ with $(\Delta S_{sc} + \Delta S_w)$ for sites A, B, and C. The slope of the line is 297 K (24 °C) and the intercept is −5 kcal mol^{-1}. The simplest interpretation of Figure 7 is that ΔH_{sc} and ΔH_w, and ΔS_{sc} and ΔS_w are separately correlated. In other words, an increased ΔH_{sc} is associated with an increased ΔH_w, and ΔS_{sc} increases with increasing ΔS_w. Proof of this idea awaits the chemical identification and states (in interactions (A, B, C)···X,Y or free) of HA sites A, B, and C.[34,38,48]

Conclusions

Adsorption of herbicides alachlor (**24**) and metalochlor (**25**) (Figure 2) on activated charcoals has adsorption enthalpies and entropies near zero[31] (Table S2),[21] which we take to indicate van der Waals (or "hydrophobic")[9] interactions with the carbon surfaces. The origin of the proposed adsorption model in Figure 5 is the need to account for the very wide range of enthalpy and entropy changes for nucleic acid constituent solute adsorption in Table S1 and Figure S1.[21] The proposed model predicts the plots in Figures 6 and 7 and thus provides an explanation for the "same underlying molecular mechanism" interpretation of the strong data correlation in Figure S1.[2,21]

The enthalpy and entropy changes associated with site "creation" and group X, Y, etc. hydration[38,48] at broken HA linkage points are negative (Table 4).

The proposed adsorption model results in the correlation shown in Figure 7 presumably because water is attached to the other functional groups X, Y, etc. released on solute adsorption (Figure 5, step e).

We have found[52] that the enthalpies and entropies of adsorption of nucleic acid constituent solutes on a different, peat-derived HA and its mercury-saturated form give a linear fit like the line in Figure S1.[2,21]

Despite success with a reasonable model that explains anomalous solute adsorption on three compost-derived HA sites A, B, and C in terms of site access and hydration, we do not know the identity of A, B, and C and we do not know the nature (physical or chemical) of the adsorption process. There is FTIR evidence for carboxylic acid and phenol involvement in reactions of adenine and cytosine with compost-derived HA.[1,2] Further insight will come from measurements of adsorption kinetics.[48] Studies with monofunctional model polymers like polyacrylic acid and poly(4-hydroxystyrene) might be useful, although such polymers are much simpler than highly functionalized HA.

Nucleic acid constituents have the properties of acids, bases, electron donors/acceptors and hydrogen-bond formers[37,53] and they are capable of chemical condensation reactions like 3−6 with the known functional groups[9−11] of humic acids. Molecular structural differences between nucleobases have dramatic effects on one or all of these properties.[37] A crucial example is the genetic code, which depends on the strong preference of adenine to pair with thymine and of guanine to pair with cytosine through multiple hydrogen bonds that link the double strand of DNA.[53]

We do not know if the kinds of interactions are the same or different when a fixed solute adsorbs at the different HA sites A, B, and C. There could be a primary interaction (say with carboxylate) and secondary interactions of the nucleobase with nearby HA constituents. Studies with simpler monofunctional solutes (for example, pyridines) should be helpful in this regard.

The least we have accomplished is to demonstrate how the interactions of solutes with complicated adsorbents like HA should be examined experimentally and how adsorption models can be suggested for refinement in future work. Establishment of temperature- and solute molecular weight-independent site capacities, maximum site capacities (eq 2), average and relative site capacities and equilibrium constants, sensible temperature dependences of equilibrium constants, and absolute and relative thermodynamic parameters gives study of adsorption on heterogeneous solids like humic acids a firm foundation. Extensive, detailed adsorption data are needed to test our model with simpler solutes and appropriate polymer models. We intend to use these tools in future work. We hope that this approach, rather than qualitative examination of isotherms under limited experimental conditions, will become the norm in future humic substances studies. The effort is necessary to fully understand HS as very important environmental materials.[9−11]

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Supporting Information Available: Data for adsorption of nucleic acid constituents on compost-derived humic acid (Table S1); site capacity and equilibrium data for adsorption of solutes on HAs (Table S2); plot of adsorption enthalpy vs adsorption entropy for adsorption of nucleic acid constituents **1**−**14** on compost-derived humic acid sites A, B, and C (data from Table S1, Figure S1) (6 pages). Ordering information is given on any current masthead page.

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(37) Nucleobases like cytosine (**1**) and nucleosides like guanosine (**13**) are neutral under the experimental conditions,[1,2] while nucleotides such as guanosine 5′-monophosphate (**14**) are monoanions: Barton, J.; Lippard, S. J. In *Nucleic acid-metal ion interactions*; Spiro, T. G., Ed.; Wiley: New York, 1980; pp 33−107. Solute charge might be important in adsorption on a membrane assembled with physical (van der Waals, hydrogen bonding) interactions between covalent HA molecules.[12]

(38) Figure 5 step c asks that A sites be freed up from interactions A−(X,Y...), with A, X, Y, etc. as different HA functional groups, by primary adsorption at free surface A sites. We assume A−A links are not involved and sites A−(X,Y...) are separated enough to accommodate newly adsorbed solutes (hence the separate zippers in Figure 5). In our model, primary adsorption at free A on the HA particle triggers an adsorption cascade near that site. The result is the value of n_A, etc. for each solute in Table 3. The cascade stops when no more A sites can be accessed for adsorption near the site of primary adsorption at A. We assume that freed groups X, Y, etc. are hydrated by water in step e of the model, which envisions the same course of events at sites B and C.

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