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Calorimetric investigation of the adsorption of nitrogen bases and nucleosides on a hydrophobic interaction sorbent

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Abstract

Heats of adsorption for nitrogen bases and nucleosides on Sepharose CL–6B, a hydrophobic interaction adsorbent, were collected through flow microcalorimetry in order to ascertain the thermodynamic driving force for adsorption in each case. It was determined that enthalpy changes associated with base stacking self-interactions can contribute significantly to the observed heats of adsorption. Accordingly, the observed heats were the net effect of the adsorbate/adsorbent interactions and the adsorbate stacking self-interactions. Since base stacking proceeds beyond the dimer stage, multi-layer adsorption of these compounds is possible, even at low solution concentrations.

Keywords: Heat of adsorption; Hydrophobic interaction chromatography; Thermodynamic parameters; Oligonucleotides

1. Introduction

Oligonucleotides are key components in a relatively new class of drugs that hold promise in combating genetic diseases as well as acquired immune deficiency syndrome (AIDS) and some forms of cancer. However, a significant technical challenge in the large-scale production of these drugs is oligonucleotide purification. Liquid chromatography has long been recognized as a powerful technique for the purification of biomolecules [1]. Diogo et al. have recently demonstrated the applicability of hydrophobic interaction chromatography (HIC) to preparative-scale oligonucleotide purification [2–4]. Their research indicated that double-stranded plasmid DNA could be separated from single-stranded nucleic acid impurities using HIC. Other purification techniques, such as displacement chromatography and membrane adsorber chromatography using ion exchange and reversed phase have also been investigated for synthetic oligonucleotides [5–8].

The study of oligonucleotide adsorption on hydrophobic adsorbents is a relatively recent development. In this paper, efforts to characterize the driving forces for adsorption of nitrogen bases and nucleosides onto Sepharose CL–6B, a weak hydrophobic adsorbent, are described. Since nitrogen bases and nucleosides are constituents of the monomers in oligonucleotides, by better understanding the nature of the adsorption of these simple molecules, hydrophobic adsorption of oligonucleotides can be systematically explored.

Some chromatographic retention data for nitrogen base and nucleoside adsorption to silica stationary phases are reported in the literature, but these were not analyzed in terms of thermodynamic parameters [9–11]. Additionally, limited isotherm data have been obtained for nucleosides adsorbing to silica from water [12], but according to Basiuk [13], no direct thermodynamic data have been presented.

Although HIC has been successfully utilized for biomolecular purification, particularly proteins, for a number of years [14], the thermodynamics of the underlying adsorption process is not well understood. In fact, the scale-up of chromatographic separation units is surprisingly unscientific, in part because of this lack of understanding [15].

Calorimetric measurements of protein adsorption on HIC supports have shown that the process is typically entropically driven. For example, Esquibel-King et al. [16] reported endothermic heats of adsorption for BSA on Sepharose CL–6B. According to Thrash and Pinto [17], the fact that adsorption is entropically driven in HIC may explain why it is generally less disruptive to biomolecular structures than other types of chromatography.

In this paper, the heats of adsorption for the nitrogen bases were compared with heats of adsorption of corresponding nucleosides to determine the effect of the sugar

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2′-deoxyribose on adsorption on the selected HIC support. Generally, the hydrophobicity of an oligomer can be determined using the following expression [18]:

$$H = \sum_{i=1}^{n} B_i n + (n-1)P \quad (1)$$

where H is the hydrophobicity of the solute, B_i the hydrophobicity of the base i, n the chain length, and P is the hydrophobicity of the polar groups. In the case of oligonucleotides, P is generally considered the hydrophobic effect of the phosphate group. According to this model, the oligonucleotide groups primarily responsible for hydrophobic interactions are the nitrogen bases and the phosphate groups. For a homo-oligonucleotide, the above expression can be rewritten as:

$$H = Bn + (n-1)P \quad (2)$$

As indicated in Eq. (2), the hydrophobicity of the nitrogen base largely determines the homo-oligonucleotide adsorption onto a hydrophobic support. Furthermore, Eq. (2) suggests that the sugar, 2′-deoxyribose, does not contribute to the hydrophobicity of the oligomer. Thus, the adsorption behavior of a base on a hydrophobic support should be essentially the same as for the corresponding nucleoside. This assumption was explored by comparing the heats of adsorption.

2. Experimental

2.1. Apparatus

Calorimetric data were gathered using a flow microcalorimeter (FMC; Gilson Instruments, Westerville, OH, USA). It operates isothermally and includes syringe micropumps to ensure precision fluid delivery. Temperature changes within the cell are detected through highly sensitive thermistors.

The FMC is equipped with an injection loop that can be reconfigured to accommodate different sample volumes. The sample is loaded into the injection loop with a syringe, and injected into the packed cell via a multiport valve. The effluent from the cell is collected and measured for the biomolecule concentration using a UV spectrophotometer (Milton Roy, Rochester, NY, USA) at a wavelength specific to the target biomolecule.

2.2. Materials and methods

The nitrogen bases adenine (Sigma product number A-8626) and thymine (T-0376) and the related nucleosides 2′-deoxyadenosine (D-7400) and thymidine (T-9250) were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Adenine and thymine were studied at a concentration of 0.25 mg/ml; 2′-deoxyadenosine and thymidine were studied at a concentration of 2.0 mg/ml. The lower concentration for bases was based on solubility considerations, while the concentration of the nucleosides was selected to correspond to concentrations used for oligonucleotides in a follow-up study. It is noted that, based on chromatographic response, both concentrations are in the linear region of the isotherm. Adenine, thymine, and thymidine solutions were dissolved at room temperature. To aid in dissolution, 2′-deoxyadenosine solutions in 20 ml glass bottles were sealed with parafilm and heated to a temperature of approximately 45 °C for about 4 h.

Crystalline Trizma buffer was purchased from Sigma. The crystals were weighed and added to deionized water to make a 10 mM buffer solution. In order to inhibit bacterial growth, 0.01% (w/w) sodium azide (Sigma), was added to the buffer solution. The buffer solution was titrated to a pH of 8.0 using HCl (Fisher Scientific Company, Hanover Park, IL, USA). Analytical grade ammonium sulfate (Fisher Scientific Company) was chosen as the modulator. After the addition of the ammonium sulfate, the solution was again titrated to pH 8.0 using NaOH from Fisher Scientific Company.

The chosen hydrophobic support, Sepharose CL–6B, was synthesized by the research group of Professor J.A. Queiroz, Universidade da Beira Interior, Portugal. It is a Sepharose derivative synthesized by covalent immobilization of the ligand 1,4-butanediol diglycidyl ether. The structure of this support is described elsewhere [16].

Sepharose CL–6B was crushed to a fine powder prior to use in the FMC. It has been previously determined that approximately 0.154 g of the adsorbent, when contacted with carrier fluid, expands to approximately 0.171 ml, the volume of the FMC cell. Accordingly, 0.154 g of the powder was mixed with approximately 1 ml of the buffer solution and allowed to swell in a covered beaker at room temperature for several days.

The cell was packed with the swelled Sepharose CL–6B powder. It was then equilibrated for several days with buffer from the syringe micropumps at flow rates ranging from 0.33 to 1.65 ml/h. When equilibrium was attained, the carrier fluid was introduced via the syringe micropumps and the system was allowed to reach thermal equilibrium.

After thermal equilibration, a 0.52 ml sample of the biomolecule dissolved in the carrier fluid was loaded into the injection loop and introduced into the packed cell via the multiport valve. The heat signal was recorded and evaluated against a calibrated heat value. This calibration value was determined by heat dissipation of a known magnitude inside the cell, with the cell packed with adsorbent and carrier fluid passing through the cell. The error estimate for the heat of adsorption was made using the method of Kline and McClintock [19].

The total amount of biomolecule in the feed and effluent were checked with a mass balance. Briefly, the concentrations of the injected sample and the effluent were measured using the UV spectrophotometer. All concentration measurements were made in triplicate. The UV wavelengths utilized

for the bases and nucleosides were as follows: 254 nm for adenine and 2′-deoxyadenosine and 264 nm for thymine and thymidine. In all cases, the mass balances demonstrated that 100 ± 5% of the injected biomolecule was recovered. This indicates that the biomolecules are eluted through the cell, identical to an isocratic elution operation in chromatography.

After each sample injection and heat measurement, the FMC was re-equilibrated with the appropriate carrier fluid to prepare for the next sample injection.

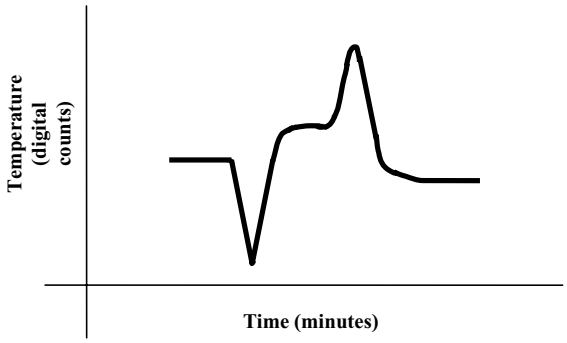

Fig. 1. Example thermogram obtained for thymidine adsorbing to and desorbing from Sepharose CL–6B. This peak was obtained at the following conditions: flow rate, 1.65 ml/h; sample, 2.0 mg/ml thymidine in 1.5 M ammonium sulfate; pH, 8.0; temperature, 26.2 °C.

3. Results

Heats of adsorption for the nitrogen bases adenine and thymine and the nucleosides 2′-deoxyadenosine and thymidine were obtained at selected ammonium sulfate concentrations between 1.0 and 2.0 M. The limited solubility of the nitrogen bases and nucleosides necessitated low solution concentrations, which correspond to linear isotherm conditions within the column.

3.1. Thymine and thymidine

For both thymine and thymidine, bimodal peaks were obtained for all ammonium sulfate concentrations. Each bimodal peak consisted of an endothermic peak followed by an exothermic peak. A typical thermogram is shown in Fig. 1. The peaks are Gaussian in shape, and do not overlap. Additionally, the areas under the endothermic peak and the exothermic peak have, within experimental error, the same magnitude in all cases. For these reasons, and because 100 ± 5% of the injected target biomolecule was recovered in all cases, it can be concluded that the endothermic peak corresponds to the adsorption peak, while the exothermic peak corresponds to the desorption peak.

The peaks were analyzed by determining the area of the peak and comparing it to a calibration peak of known magnitude, as previously discussed. The observed heat of adsorption values for thymine and thymidine are shown in Figs. 2 and 3, respectively.

For thymine, the observed heats of adsorption increase nearly linearly with increasing ammonium sulfate concentration. This behavior is consistent with results cited in the literature for weak hydrophobic interactions in the presence of ammonium sulfate [14,16]. Similarly, the observed heats of adsorption for thymidine also generally increase as the ammonium sulfate concentration increases. Although the heat of adsorption for thymidine at 1.7 M ammonium sulfate is slightly greater than that at 2.0 M, the value is within experimental error, suggesting that the heats of adsorption at 1.7 and 2.0 M are approximately equal.

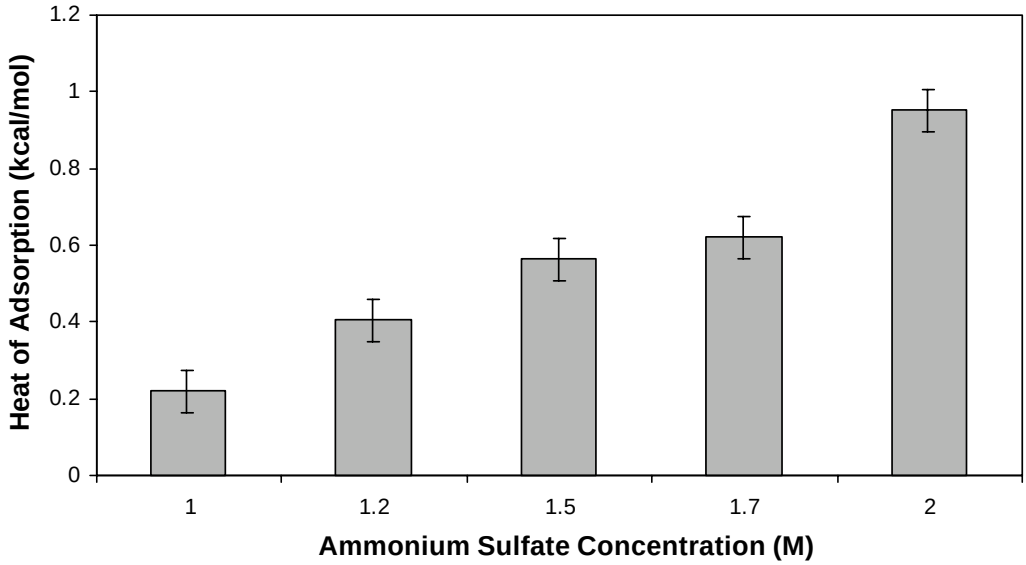

Fig. 2. Observed heats of adsorption for thymine on Sepharose CL–6B at varying ammonium sulfate concentrations. Data were collected at 26 ± 1.0 °C, a flow rate of 1.65 ml/h, a solution pH of 8.0, and a thymine concentration of 0.25 mg/ml.

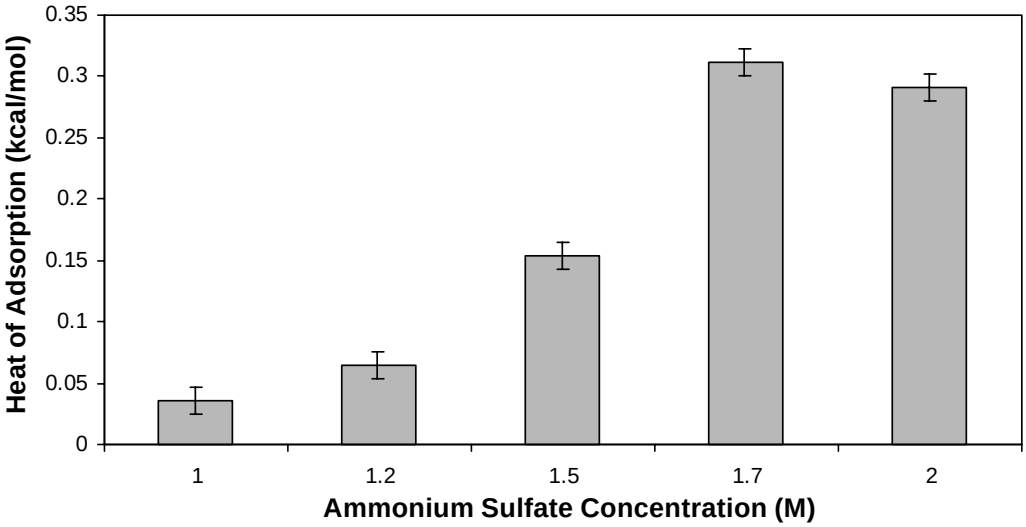

Fig. 3. Observed heats of adsorption for thymidine on Sepharose CL–6B at varying ammonium sulfate concentrations. Data were collected at 26 ± 1.0 °C, a flow rate of 1.65 ml/h, a solution pH of 8.0, and a thymidine concentration of 2.0 mg/ml.

3.2. Adenine and 2′-deoxyadenosine

As with the thymine and thymidine peaks, the adenine peaks were bimodal with an endothermic peak followed by an exothermic peak. The peaks are Gaussian in shape, indicating that they do not overlap, and magnitudes of the endothermic and exothermic peaks are approximately equal. Thus, as with the thymine and thymidine peaks, it was concluded that the endothermic peak corresponds to the adsorption peak and the exothermic peak corresponds to the desorption peak.

The observed heats of adsorption for adenine are shown in Fig. 4. The heats of adsorption decrease slightly with increasing ammonium sulfate concentration. This behavior is the opposite of that cited in the literature for weak hydrophobic interactions in the presence of ammonium sulfate [14,16].

Data obtained for 2′-deoxyadenosine were quite different from that of the other three solutes. The 2′-deoxyadenosine peaks were bimodal with the exothermic peak followed by the endothermic peak. Furthermore, the shape of the exothermic peak is skewed, as shown in Fig. 5, suggesting an overlap of separate effects progressing at different rates. Mass balances indicated that, as with the other three solutes, all the solute injected was recovered within approximately 100 ± 5%.

The observed effect of salt on heats of adsorption for 2′-deoxyadenosine are shown in Fig. 6. In sharp contrast to the other solutes, for 2′-deoxyadenosine the heats of adsorption are exothermic, and become more so with increasing ammonium sulfate concentration.

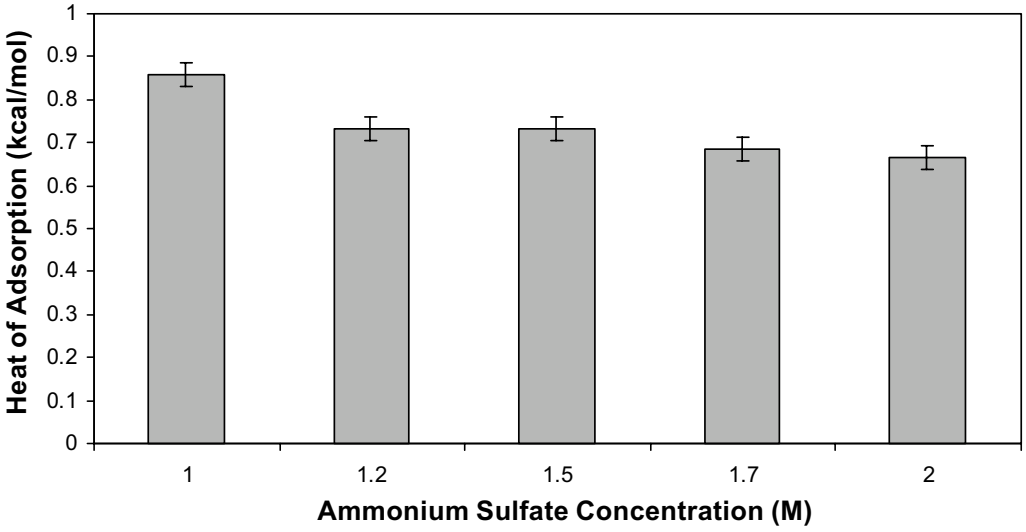

Fig. 4. Observed heats of adsorption for adenine on Sepharose CL–6B at varying ammonium sulfate concentrations. Data were collected at 26 ± 1.0 °C, a flow rate of 1.65 ml/h, a solution pH of 8.0, and an adenine concentration of 0.25 mg/ml.

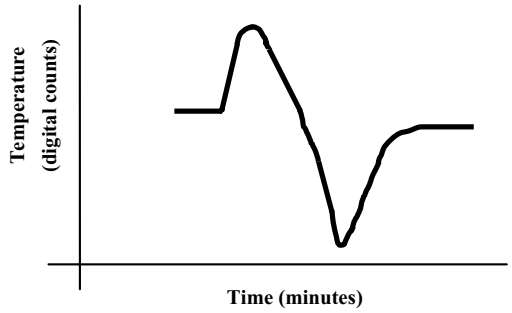

Fig. 5. Example peak obtained for 2′-deoxyadenosine adsorbing to and desorbing from Sepharose CL–6B. This peak was obtained at the following conditions: flow rate, 1.65 ml/h; sample, 2.0 mg/ml 2′-deoxyadenosine in 1.7 M ammonium sulfate; pH, 8.0; temperature, 26.2 °C.

4. Discussion

The driving force for adsorption due to weak hydrophobic interactions is considered to be the increase in entropy that results with the release of water molecules from both the biomolecule and the surface of the adsorbent [1,16,20]. An enthalpically unfavorable effect, manifested through an endothermic heat of adsorption, is overcome by a large positive entropy change due to water release. As stated above, endothermic heats of adsorption were, in fact, observed for three of the four solutes studied here: thymine, thymidine, and adenine.

Figs. 2 and 3 demonstrate that the observed endothermic heat of adsorption increases with increasing ammonium sulfate concentration for both thymine and thymidine. These data are consistent with results reported in the literature for biomolecules adsorbing onto weak hydrophobic adsorbents [16,21]. As the ammonium sulfate concentration increases, hydrophobic interactions between biomolecules and weak hydrophobic adsorbents increase because the surface tension of the carrier fluid increases [22].

According to Lin et al. [23], adsorption of a protein to a hydrophobic adsorbent can be divided into five subprocesses: exclusion of water molecules or ions from the protein surface, exclusion of water molecules or ions from the adsorbent surface, hydrophobic interactions between the protein and adsorbent, structural rearrangement of the protein upon adsorption, and structural rearrangement of the excluded water molecules or ions into the bulk solvent. Similarly, the adsorption of nitrogen bases and nucleosides to a hydrophobic adsorbent may be considered in terms of the same subprocesses. For thymine and thymidine adsorption onto Sepharose CL–6B, the observed endothermic heats of adsorption probably derive from a combination of biomolecule dehydration, surface dehydration, and hydrophobic interaction. Norde [24] demonstrated that the free energy associated with dehydration that accompanies protein adsorption onto solid surfaces ranges between 5 and 12 mJ/m^2. Clearly, a relatively large endothermic contribution due to dehydration, coupled with a smaller exothermic contribution due to hydrophobic interaction, results in a small net endothermic heat of adsorption. Furthermore, because these molecules have a relatively inflexible structure and are very small in comparison with other biomolecules commonly purified through chromatography, i.e., proteins, endothermic effects due to structural rearrangement of nitrogen bases and nucleosides upon adsorption are negligible.

In the case of adenine, the observed heat of adsorption decreases with increasing ammonium sulfate concentration. This behavior is opposite to that reported in the literature for solutes undergoing hydrophobic interactions with an increase in salt concentration [16,21]. Additionally, the observed heats of adsorption for 2′-deoxyadenosine are exothermic, and become more exothermic with increasing ammonium sulfate concentration. The thermodynamic data collected for adenine and 2′-deoxyadenosine can be explained when the effect of base stacking is taken into

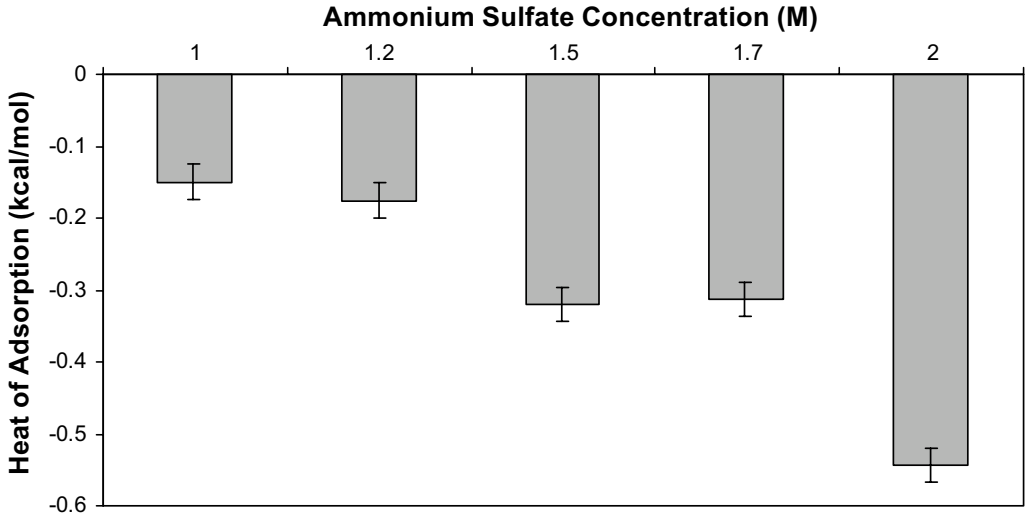

Fig. 6. Observed heats of adsorption for 2′-deoxyadenosine on Sepharose CL–6B at varying ammonium sulfate concentrations. Data were collected at 26 ± 1.0 °C, a flow rate of 1.65 ml/h, a solution pH of 8.0, and a 2′-deoxyadenosine concentration of 2.0 mg/ml.

account. It is probable for several reasons that each of these measured heats of adsorption is actually the composite of the heat of adsorption and the heat associated with molecular base stacking.

In the 1960s several research groups studied the interactions between purines, pyrimidines, and nucleosides in solution. Ts'o and coworkers reported in a series of papers that nitrogen bases and nucleosides not only interact heterogeneously in aqueous solutions, but also self-associate. They concluded that the interactions between these molecules are not due to lateral hydrogen bonding but base stacking [25–30]. Furthermore, it was determined that these interactions proceed beyond the dimer stage, creating stacks containing numerous bases [25]. In fact, the base stacking of these molecules is much like that observed in oligonucleotides and DNA. According to Solie and Schellman [31], "the asymmetric stacking is the stablest form of interaction and is not forced upon the system by the presence of the sugar–phosphate chain", which connects the monomers in DNA and RNA.

Thermal osmometry studies have shown association constants for thymidine and 2′-deoxyadenosine to be in the range of 0.91 and 4.7–7.5 m^{-1}, respectively [20]. The changes in free energies associated with base stacking were then calculated and determined to be 0.060 kcal/mol (1 cal = 4.184 J) for thymidine and between −0.92 and −1.195 kcal/mol for 2′-deoxyadenosine [30]. Through microcalorimetry, Gill et al. [32] determined that both enthalpic and entropic effects in self-association were negative, and both contributed significantly to the free energy change. Ts'o [33] utilized the calorimetric data of Gill et al. [32] and Farquhar et al. [34] to determine the change in enthalpy due to self-association for thymidine and deoxyadenosine, and reported values of -2.4 ± 0.3 and -6.5 ± 1.0 kcal/mol, respectively. Unfortunately, the corresponding self-association enthalpic changes for thymine and adenine have not been studied, though the trend of decreasing ΔH_{ads} with increasing ionic strength for adenine suggests that these enthalpic contributions may not be negligible for all bases. In the case of adenine it is postulated, based on results in the literature that will be discussed later, that base stacking effects, which lead to exothermic heats, increase with an increase in salt concentration. However, the adsorbate/adsorbent interaction dominates the overall process to give the net endothermic heats of adsorption reported in Fig. 4.

The mechanism for base stacking was initially considered to be primarily hydrophobic interactions because the phenomenon was observed only in aqueous solutions [35]. However, it was concluded that bases and nucleosides self-interact due to a combination of hydrophobic interactions, Van der Waals forces, and π-electron interactions [26]. Clearly a combination of these forces would, in fact, result in significant enthalpic and entropic contributions to the overall free energy change.

The base stacking studies cited above were all performed in aqueous solution, and it was observed that this phenomenon occurs at relatively high solute concentrations. These results raise the possibility that base stacking also occurs when nitrogen bases and nucleosides are adsorbed on chromatographic supports, i.e., in the adsorbed phase. In this case, surface concentrations of these materials can be high, particularly in regions of the adsorbent that are easily accessible to the solute. This implies that the calorimetric peaks observed in this study may be due to a combination of adsorbate/adsorbent interactions and base stacking.

The adsorption process can be conceptualized as follows. Solute initially adsorbs on the surface of the adsorbent and subsequently can either base stack or adsorb on the adsorbent surface. As previously mentioned, base stacking can proceed beyond the dimer stage, so the number of bases in any given stack would not hinder further stacking of incoming solute. At solution concentrations used in the calorimetric measurements of this study, base stacking in solution is unlikely, and solute adsorbs in monomolecular form.

The high association constant of 2′-deoxyadenosine suggests that its affinity to self-interact may be as strong as its affinity to adsorb to the selected adsorbent, or even more so. In fact, all reported values for the self-association constant of deoxyadenosine are at least four times that of thymidine [26,31]. This high association constant could also explain why skewed peaks were obtained for 2′-deoxyadenosine but not for the other three compounds studied. As previously mentioned, skewed peaks result from a combination of processes occurring concurrently, but at different rates. For thymine, adenine, and thymidine, the peaks are Gaussian in shape, either indicating that one process dominates overall, or that the rates of all processes are similar. The data suggest that base stacking is not negligible for thymine, adenine, and thymidine, as will be discussed later. Very significantly, however, base stacking effects for 2′-deoxyadenosine appear to be as significant as adsorption effects. This observation is supported by the fact that the self-association constant for 2′-deoxyadenosine is significantly greater than that for thymidine.

All the peaks obtained for 2′-deoxyadenosine were exothermic, which is uncharacteristic for weak hydrophobic interactions in the presence of ammonium sulfate. Although exothermic heats of adsorption have been observed in the case of strong attractive forces between the adsorbate and adsorbent, there is no evidence that such strong interactions occur in this case. It is expected that the hydrophobicity of adenine and 2′-deoxyadenosine are approximately equal, since the nitrogen base provides the primary hydrophobic contribution. As discussed earlier, the strength of a hydrophobic interaction is largely determined by the hydrophobicity of the adsorbing molecule. 2′-Deoxyadenosine only differs from adenine in that it contains the sugar 2′-deoxyribose. If the addition of the 2′-deoxyribose to the base caused the adsorbate/adsorbent interaction to become strongly attractive, it would be expected that heat of adsorption trends for 2′-deoxyadenosine and thymidine, in relation to heat of adsorption trends for their related nitrogen

Table 1
A comparison of the differences in the heats of adsorption for thymine and thymidine with differences in the heats of adsorption for adenine and 2′-deoxyadenosine

$(NH_4)_2SO_4$ concentration (M)	ΔQ_{ads} (kcal/mol)	
	Thymine–thymidine	Adenine–2′-deoxyadenosine
1.0	0.18	1.0
1.2	0.34	0.9
1.5	0.41	1.1
1.7	0.31	1.0
2.0	0.66	1.2

bases, would be similar. In fact, our data indicate that the differences in the heats of adsorption for adenine and 2′-deoxyadenosine are significantly greater than those for thymine and thymidine, as shown in Table 1. While this observation does not discount the possibility that the addition of the 2′-deoxyribose changes the adsorption characteristics to some degree, clearly the addition of the sugar group does not fully explain the exothermic heats of adsorption obtained for 2′-deoxyadenosine. Accordingly, these data indicate that the large discrepancy in the 2′-deoxyadenosine peaks and the adenine peaks is due to at least one effect besides interactions with the surface.

Because the reported change in enthalpies of base stacking are on the same order of magnitude as most of the observed heats of adsorption, and because those changes are exothermic and hydrophobic interactions between weak hydrophobic adsorbents and biomolecules typically produce endothermic heats of adsorption, it is reasonable to hypothesize that the observed heats of adsorption are the net effect of a negative enthalpy change due to base stacking and a positive enthalpy change due to adsorbate/adsorbent interaction. As previously stated, the relative contributions of these two effects are determined by the relative affinities for the adsorbent surface and for self-association.

Further support for the hypothesis that the enthalpy change of base stacking significantly contributes to the overall observed heat of adsorption comes from an examination of the adenine and 2′-deoxyadenosine data. It has been reported that base stacking self-interactions vary with salt concentration [36]. Robinson and Grant [37] found that self-interaction effects in 2 M salt solutions were up to 50% greater than those in 1 M salt solutions. The fact that self-interactions increase with increasing salt concentration explains the decrease in observed heats of adsorption for adenine and 2′-deoxyadenosine with increasing salt concentration. If we view the observed heat of adsorption as a sum of the enthalpy change for adsorbate/adsorbent interactions and the enthalpy change of base stacking, it is possible that the observed heat of adsorption may actually decrease with salt concentration. According to Scoble [38], changing the ionic strength of the mobile phase only slightly affects the retention behavior of nucleosides and bases in HIC. This implies that the adsorption characteristics of these biomolecules would also change only slightly with a change in mobile phase salt concentration. Coupling a slightly increasing endothermic heat of adsorption with a more strongly increasing exothermic heat of base stacking as salt concentration increases, will result in a decrease in the observed heat of adsorption. This is, in fact, the trend observed for adenine and 2′-deoxyadenosine.

Since the association constant and the enthalpy change of base stacking for thymidine are significantly smaller than those for 2′-deoxyadenosine, it is likely that base stacking makes a smaller contribution to the observed heats of adsorption for thymidine. A comparison of the thymine and thymidine data reveals that all observed heats are endothermic, and they increase slightly with increasing salt concentration. In addition, the magnitudes of the endothermic peaks for thymidine are consistently smaller than those for thymine. This relatively small discrepancy in heats of adsorption for thymine and thymidine could be due to: the addition of the 2′-deoxyribose, as previously discussed; the differences in the base stacking self-interactions of thymine and thymidine; or a combination of both. However, the fact that the observed heats of adsorption for both thymine and thymidine increase with increasing ammonium sulfate concentration (as is expected for weak hydrophobic interactions) suggests that the contribution of adsorption to enthalpy change is more significant than that of base stacking for these compounds.

Combining the fact that the enthalpy change of base stacking is significantly larger for deoxyadenosine than thymidine with the observation that the observed heats of adsorption for 2′-deoxyadenosine are exothermic while those for thymidine are endothermic indicates that, indeed, enthalpic contributions due to self-interactions cannot be ignored. Furthermore, the differences between the observed heats of thymine versus thymidine and adenine versus 2′-deoxyadenosine could be entirely due to differences in their respective enthalpy change of base stacking.

As discussed previously, the hydrophobicity of a homo-oligonucleotide is given by Eq. (2) [18]. An assumption of this equation is that the effect of the sugar, in this case 2′-deoxyribose, is not significant for calculating the hydrophobicity of an oligonucleotide. This assumption was examined by comparing the heats of adsorption, which are assumed to correlate proportionally with the hydrophobicity, for two nitrogen bases and their related nucleosides, specifically thymine with thymidine and adenine with 2′-deoxyadenosine. The results of this study do not indicate that the addition of 2′-deoxyribose to the nitrogen base significantly affects the hydrophobicity of the molecule. The differences observed between the heats of adsorption for the nitrogen bases and their related nucleosides can be attributed to base stacking self-interaction effects that occur with nitrogen bases and nucleosides, but not with oligonucleotides. Thus, the assumption in Eq. (2) that the sugar does not contribute to the hydrophobicity of an oligonucleotide cannot be contested. However, the addition of the

sugar clearly alters other important properties related to the adsorption of nitrogen bases and nucleosides, such as the solubility and the propensity to base stack.

5. Summary and conclusions

Heats of adsorption for the nitrogen base thymine and the nucleoside thymidine on the HIC support are endothermic and increase with increasing ammonium sulfate concentration. For the nitrogen base adenine, heats of adsorption are endothermic and decrease with increasing ammonium sulfate concentration. Heats of adsorption for the nucleoside 2′-deoxyadenosine are exothermic and become increasingly exothermic with an increase in ammonium sulfate concentration.

These microcalorimetric data reveal that the adsorption behavior of nitrogen bases and nucleosides on an HIC support in aqueous solution is a complex phenomenon. Although hydrophobic interactions appear to be the primary mechanism for adsorption to the hydrophobic adsorbent, the biomolecules exhibit base stacking behavior on the adsorbent surface even at low solution concentrations. Accordingly, it was concluded that the measured heats of adsorption represented the net effect of two different types of interactions: adsorbate/adsorbent interactions and base stacking self-interactions between like molecules. Furthermore, the relative contributions of these two effects are determined by the relative affinities of the biomolecule for the adsorbent surface and for self-association.

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