Thermodynamics of Oligosaccharide Binding to a Monoclonal Antibody Specific for a *Salmonella* O-Antigen Point to Hydrophobic Interactions in the Binding Site*

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The thermodynamic characteristics of oligosaccharide binding to an antibody binding site that is dominated by aromatic amino acids suggest that the hydrophobic effect contributes substantially to complex formation as well as hydrogen bonding and van der Waals interactions. A detailed titration microcalorimetric study on the temperature dependence of the binding of a trisaccharide, representing the epitope of a *Salmonella* O-antigen, showed that its maximum binding to the monoclonal antibody Se155-4 occurs just below room temperature and both enthalpy and entropy changes are strongly dependent on temperature in a mutually compensating manner. The heat capacity change also shows an unusually strong temperature dependence being large and negative above room temperature and positive below. van’t Hoff analysis of the temperature dependence of the binding constant yielded a biphasic curve with two apparent intrinsic temperature dependence of the binding constant

\[ \Delta H^0 \text{ versus } -T\Delta S^0, \]

observed for temperature-dependent measurements mirror the behavior seen for a series of functional group replacements, suggesting that the molecular and physical origin of this phenomena are closely related and linked to the role of water in complex formation. The thermodynamic results are compared to the mode of binding determined from a 2.06-Å resolution structure of the Fab-oligosaccharide complex, and with literature data for the heat capacities of sugars in aqueous solution and for the thermodynamics of carbohydrate binding to transport proteins and lectins.

The study of the interactions between antigenic bacterial surface polysaccharides and specific antibodies represents an area of the important field of protein-carbohydrate interactions (1–3) which also includes the study of lectins (4–8), sugar transporting proteins (9–10), and carbohydrate processing enzymes (11–13). In order to develop a deeper understanding of the interactions between these carbohydrate antigens and antibodies, the use of synthetic or modified O-antigenic haptenso and monoclonal antibodies has proven very useful (9). One surface polysaccharide from a pathogen of particular interest is the *Salmonella* serogroup B O-antigen, which has a chemical structure characterized by a branched repeating unit with four sugar residues: (\(-3\))-\(\alpha\)-D-Galp(\(1\rightarrow2\))-[\(\alpha\)-D-Abe]\(\alpha\)-D-Manp(\(1\rightarrow4\))-\(\alpha\)-L-Rhap(\(1\rightarrow\))], in which Abe stands for abequose (3, 6-dideoxy-\(\alpha\)-D-hexose) and Rha stands for rhamnose (6-deoxy-mannose). A murine monoclonal antibody, designated Se155-4, raised against this antigen recognizes the first three sugar residues of one repeating unit as its epitope (\(-3\))-\(\alpha\)-D-Galp(\(1\rightarrow2\))-([\(\alpha\)-D-Abe]\(\alpha\)-D-Manp)(\(1\rightarrow4\))-\(\alpha\)-L-Rhap(\(1\rightarrow\))]. We have previously reported a thermodynamic study of the chain length dependence of the binding of haptons by this antibody (15) and the three-dimensional structure of the Fab complexed with a dodecasaccharide hapten (three repeating units of the O-antigen) (14). Furthermore, nuclear magnetic resonance spectroscopy and theoretical calculations have provided a three-dimensional model of the O-antigen\(^1\) (16, 17), and synthetic genes coding for a fully functional Fab and single chain Fv have been expressed in *Escherichia coli* (18–19).

The specific recognition of oligosaccharides by proteins such as antibodies, lectins, enzymes, and transport proteins is characterized by binding constants in the range 10\(^4\) to 10\(^8\) M\(^{-1}\) and is a remarkable process since carbohydrates are generally considered to be hydrophilic and highly solvated. To date there have been few, if any, studies of such binding using the accurate technique of titration microcalorimetry. In the appropriate range this technique offers the potential to directly determine the binding constant \(K\) and the molar enthalpy \(\Delta H^0\) and thereby also \(\Delta G^0\) and \(\Delta S^0\) from a single titration experiment (15). Detailed thermodynamic characterization of carbohydrate antibody complexes are needed to shed light on the often incompatible contemporary interpretations of the driving force for complex formation and its stabilization, and in this paper we wish to expand the thermodynamic description of this system by reporting on the temperature dependence of the binding of the methyl glycoside of the epitope (methyl 2-\(\alpha\)-O-(\(\alpha\)-galactopyranosyl)-3-O-(\(\alpha\)-D-3,6-dideoxy-\(\alpha\)-L-hexopyranosyl)-\(\alpha\)-D-mannopyranoside) (Gal[Abe]Man)\(^2\) by this antibody.

**Experimental Procedures**

The monoclonal antibody Se155-4 (subclass IgG\(_1\)(\(\lambda\))) was obtained from mouse ascites fluid and purified by affinity chromatography.

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1 D. R. Bundle, J.-R. Brisson, S. Gagné, and H. Baumann, manuscript in preparation.

2 The abbreviation used is: Gal[Abe]Man, methyl 2-\(\alpha\)-O-(\(\alpha\)-galactopyranosyl)-3-O-(\(\alpha\)-D-3,6-dideoxy-\(\alpha\)-L-hexopyranosyl)-\(\alpha\)-D-mannopyranoside.
The binding of Gal[Abe]Man by Se155-4 was studied by titration microcalorimetry (21) at various temperatures, and the results are summarized in Table I. The free energy of binding does not vary greatly with temperature. However, the changes in entropy become increasingly unfavorable for binding at lower and higher temperatures. The antibody-hapten association is as strong and shows an entropy-compensation plot (\(-\Delta H^a\) versus \(-T\Delta S^a\)). Despite the non-monotonic temperature dependence of \(\Delta H^a\) and \(\Delta S^a\), the points lie on a nearly straight line with a slope close to unity (0.92 ± 0.11) and an intercept of \(\Delta H^a = -29.5 \pm 1.7 \text{ kJ mol}^{-1}\), where the errors represent three standard deviations from the linear regression analysis. Since the temperature dependences of both \(\Delta H^a\) and \(\Delta S^a\) are related to the change in heat capacity (Equation 1), a certain degree of compensation between these two thermodynamic functions will always be observed in temperature-dependent experiments. A linear relationship between enthalpy and entropy can be expressed by the following equation,
\[
\Delta H^a = -aT\Delta S^a + b,
\]
where \(a\) and \(b\) are constants. Differentiation of Equation 2 with respect to \(T\) and combination with Equation 1 gives the following equation.
\[
\frac{\Delta S^a}{\Delta C_p} = \frac{1}{a} - 1
\]
Hence, linearity is observed when the ratio between \(\Delta S^a\) and \(\Delta C_p\) is constant. The importance and constancy of this ratio were first pointed out by Sturtevant (23). A unity slope (complete compensation) occurs when \(\left|\Delta C_p^a\right| \gg \left|\Delta S^a\right|\) (24). In other words, the cause of the enthalpy-entropy compensation is the large change in the heat capacity compared with the change in entropy. If linearity is observed in a compensation plot, and if the slope \(a\) is significantly different from unity, then \(\Delta C_p^a\) can be estimated from Equation 3. This applies not only to temperature-dependent compensation plots, but also to isothermal enthalpy-entropy compensation (vide infra).

The near unity slope and the intercept of \(-29.5 \text{ kJ mol}^{-1}\) (the value of \(\Delta H^a\) when \(-T\Delta S^a\) is zero) are very close to the values obtained in many isothermal systems in which the compensating enthalpies and entropies characterize different structural analogues (5, 25, 26). In a recent study in our laboratory of the binding of deoxy derivatives of Gal[Abe] Man as well as mono- (Abe) and disaccharides (AbeMan) by Se155-4 at 25 °C, an isothermal enthalpy-entropy compensation plot showed near linearity for most compounds, but with a slope significantly different from unity (0.58) and an intercept of \(\Delta H^a = -26.6 \text{ kJ mol}^{-1}\). Since \(\frac{d(\Delta C_p^a)}{dT} = -\Delta S^a\), the free energy has extrema at temperatures where \(\Delta S^a = 0\), i.e. enthalpy-intercepts from compensation plots should ideally represent the largest magnitudes in free energy. It seems intriguing that the change in enthalpy when the entropy change is zero should be virtually the same for the

\[^{2}\text{D. R. Bundle and B. W.Sigurskjd, unpublished results.}\]

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**Table I**

Thermodynamics of the binding of the epitope \(\alpha-D-Galp(1 \rightarrow 2)-[\alpha-D-Abep(1 \rightarrow 3)]-\alpha-D-Manp-Ome\) by monoclonal antibody Se155-4 specific for Salmonella serogroup B O-antigen at pH 8.0 determined by titration microcalorimetry

<table>
<thead>
<tr>
<th>(T)</th>
<th>(10^3 K)</th>
<th>(\Delta G^a)</th>
<th>(\Delta H^a)</th>
<th>(-T\Delta S^a)</th>
<th>(\Delta S^a)</th>
<th>(\Delta C_p^a)</th>
<th>(K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>282.77</td>
<td>1.3 ± 0.5</td>
<td>-27.7 ± 0.8</td>
<td>-31.8 ± 2.2</td>
<td>4.0 ± 2.6</td>
<td>-14.3 ± 9.3</td>
<td>1470 ± 1000</td>
<td>284.41</td>
</tr>
<tr>
<td>286.10</td>
<td>1.6 ± 0.7</td>
<td>-28.5 ± 1.0</td>
<td>-26.9 ± 2.5</td>
<td>-1.6 ± 2.7</td>
<td>5.6 ± 5.1</td>
<td>340 ± 760</td>
<td>288.47</td>
</tr>
<tr>
<td>290.83</td>
<td>5.1 ± 3.2</td>
<td>-31.8 ± 0.9</td>
<td>-25.3 ± 2.6</td>
<td>-6.5 ± 2.7</td>
<td>22.4 ± 9.4</td>
<td>640 ± 410</td>
<td>294.57</td>
</tr>
<tr>
<td>298.31</td>
<td>2.1 ± 0.3</td>
<td>-30.5 ± 0.7</td>
<td>-20.5 ± 1.7</td>
<td>-10.0 ± 0.8</td>
<td>33.5 ± 2.6</td>
<td>-4940 ± 920</td>
<td>301.19</td>
</tr>
<tr>
<td>304.06</td>
<td>0.68 ± 0.06</td>
<td>-28.1 ± 0.2</td>
<td>-48.9 ± 5.0</td>
<td>20.8 ± 5.0</td>
<td>-68.4 ± 16.3</td>
<td>-1470 ± 980</td>
<td>307.06</td>
</tr>
<tr>
<td>310.06</td>
<td>0.47 ± 0.09</td>
<td>-27.7 ± 0.5</td>
<td>-57.7 ± 3.2</td>
<td>30.0 ± 3.5</td>
<td>-96.6 ± 11.2</td>
<td>-1470 ± 980</td>
<td>307.06</td>
</tr>
</tbody>
</table>

* Calculated as \(\Delta H^a/\Delta T\).
* Uncertainties represent three standard deviations from regression analysis.

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(20) The trisaccharide epitope Gal[Abe]Man was synthesized by Dr. A. Ragauskas, and the procedure will be described elsewhere.

Calorimetry was carried out on an OMEGA titration microcalorimeter from MicroCal, Inc. (Northampton, MA). This instrument has been described in detail by Wiseman et al. (21). An ~50 μM solution of antibody in 50 mM Tris pH 8.0, was titrated with a 2 mM solution of Gal[Abe]Man in the same buffer at various temperatures. All solutions were thoroughly degassed by stirring under vacuum. The titrations were carried out by injecting 20 portions of 5 μl of the ligand solution at 5-min intervals into the calorimeter cell from a 100-μl syringe stirring at 400 rpm. The reference cell was filled with water, and the calorimeter was calibrated by standard electrical pulses. The peaks of the obtained thermograms were integrated using the ORIGIN software supplied with the instrument by MicroCal, Inc., and the resulting isotherms were fitted by nonlinear regression as described previously (15) yielding estimates of the binding constant \(K\), \(\Delta G^a\), \(\Delta H^a\), and \(\Delta S^a\), as well as the concentration of antibody binding sites.

**RESULTS AND DISCUSSION**

The binding of Gal[Abe]Man by Se155-4 was studied by titration microcalorimetry (21) at various temperatures, and the results are summarized in Table I. The free energy of binding does not vary greatly with temperature. However, both the enthalpy and the entropy changes show strong and mutually compensating temperature dependence. The enthalpic interactions appear to go through a minimum around room temperature and become stronger both at lower and higher temperatures. The antibody-hapten association is assisted by favorable entropy contributions at room temperature, but the changes in entropy become increasingly unfavorable for binding at lower and higher temperatures. The maximum binding in terms of the largest binding constant occurs just below 290 K. Although the change in heat capacity, \(\Delta C_p^a\), is difficult to calculate with accuracy from the present data, it is clear from examination of the enthalpy values in Table I that \(\Delta C_p^a\) is also strongly temperature-dependent and changes sign from negative above 298 K to positive below this temperature. Estimates of the heat capacity changes are included in Table I at intermediate temperatures. A different temperature dependence has been found for the dextran-specific monoclonal IgM MOPC-104E in a calorimetric study ranging in temperature from 277 to 298 K (22). These workers observed a near constant and positive \(\Delta C_p^a \approx 1260 \text{ J mol}^{-1} \text{ K}^{-1}\), the maximum binding occurring at 277 K with a modest decrease in binding with increasing temperature resulting from compensating changes in enthalpy and entropy.

*Enthalpy-Entropy Compensation*—Fig. 1 shows an enthalpy-entropy compensation plot (\(-\Delta H^a\) versus \(-T\Delta S^a\)).
isothermal situation with different ligands as for the temperature-dependent situation with one ligand. This may merely reflect, however, that the experiments that show isothermal compensation have been carried out at a temperature (usually 298 K) close to the temperature where the free energy has its maximum. In spite of this, the similarity between isothermal or "structural" enthalpy-entropy compensation on the one hand, and temperature-dependent compensation on the other, still suggests that the molecular and physical origins of the two types of enthalpy-entropy compensation are closely related. The link between these two compensation phenomena is probably the large change in heat capacity compared with the change in entropy.

Hydrophobic Interactions—A large negative heat capacity change is usually taken as an indicator of a prominent hydrophobic effect, which is defined as the tendency of hydrophobic surfaces "sticking together" in water under the exclusion of solvent molecules (27–34). This definition does not include any subsequent formation of van der Waals interactions between the surfaces. A large negative heat capacity change can also arise from changes in ionization and conformational changes as well. Hydrogen bonds and other polar interactions are generally believed not to cause large changes in heat capacity. There seems to be a very good correlation between the magnitude of the heat capacity change and the area of exposed non-polar surfaces, \( \Delta A_{np} \), in water at 25 °C both for the transfer of hydrocarbons in water at infinite dilution to the pure liquid state (\( \Delta C_p^0/\Delta A_{np} = -1.35 \pm 0.38 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2} \)) and for the folding of proteins (\( \Delta C_p^0/\Delta A_{np} = -1.05 \pm 0.13 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2} \)) (34). Investigations of the thermodynamics of carbohydrates are not numerous (35–41), and no work on the temperature dependence of the change in heat capacity upon dissolution of sugars in water seems to have been reported. A \( \Delta C_p^0/\Delta A_{np} \) value for sugars can be estimated from hydrophobic indices of some monosaccharides (42) and reported differences between the heat capacity in the solid state and the partial molar heat capacity in infinitely diluted aqueous solution (38). These literature data are summarized in Table II. There does not seem to be a good internal correlation between these values; however, an average value of \( \Delta C_p^0/\Delta A_{np} = -1.02 \pm 0.14 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2} \) at 25 °C can be estimated for these simple sugars and this value corresponds very well with the values found for hydrocarbons and proteins. This would suggest that sugars indeed expose a significant area of hydrophobic surface in aqueous solution (43) and that water molecules interact with this non-polar surface in much the same fashion as with hydrocarbons. On the other hand, the unusually strong temperature dependence of the heat capacity change for the antibody-carbohydrate system under investigation is very different from the modest temperature dependence observed in hydrocarbon-water and protein folding interactions (34, 44).

It can be ruled out that changes in ionization occur to a significant extent in this system, because an experiment carried out at 25 °C in a phosphate buffer instead of Tris buffer gave identical results (data not shown). Since phosphate has a much smaller heat of ionization (5.10 kJ mol\(^{-1}\)) than Tris (48.16 kJ mol\(^{-1}\)) (see Ref. 45 and references therein), any change in the net ionization would have been expressed as a significantly different observed enthalpy in the two buffers. However, there does seem to be a significant conformational change of the hapten upon binding (14). The torsion angle of the glycosidic linkage between the galactose and mannose units is shifted about 40° from the predicted value which by crude estimates amounts to a 14 kJ mol\(^{-1}\) increase in energy from the calculated global minimum conformer (16). A possible temperature dependence of the solution conformation of the ligand could explain the observed temperature dependences of the thermodynamic functions, but so far NMR data do not support this hypothesis. There may be a multitude of different interactions, each with unique temperature depend-

### Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>( % )</th>
<th>( \Delta A_{np}^o )</th>
<th>( \Delta A_{np} )</th>
<th>( \Delta C_p^o/\Delta A_{np} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Glucose</td>
<td>36</td>
<td>97.1</td>
<td>95.8</td>
<td>-107</td>
</tr>
<tr>
<td>( \beta )-Glucose</td>
<td>64</td>
<td>95.1</td>
<td>99.0</td>
<td>-107</td>
</tr>
<tr>
<td>( \alpha )-Galactose</td>
<td>36</td>
<td>100.6</td>
<td>98.1</td>
<td>-121</td>
</tr>
<tr>
<td>( \alpha )-Mannose</td>
<td>68</td>
<td>97.1</td>
<td>98.4</td>
<td>-121</td>
</tr>
<tr>
<td>( \beta )-Mannose</td>
<td>32</td>
<td>101.1</td>
<td>95.2</td>
<td>-90</td>
</tr>
<tr>
<td>( \alpha )-Arabinose</td>
<td>63</td>
<td>100.7</td>
<td>93.5</td>
<td>-97</td>
</tr>
<tr>
<td>( \beta )-Arabinose</td>
<td>34</td>
<td>93.3</td>
<td>93.5</td>
<td>-97</td>
</tr>
<tr>
<td>( \alpha )-Xylose</td>
<td>33</td>
<td>94.8</td>
<td>93.5</td>
<td>-97</td>
</tr>
<tr>
<td>( \beta )-Xylene</td>
<td>67</td>
<td>92.8</td>
<td>94.5</td>
<td>-84</td>
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<tr>
<td>( \alpha )-Ribopyranose</td>
<td>20</td>
<td>105.2</td>
<td>101.2</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Ribopyranose</td>
<td>56</td>
<td>94.5</td>
<td>94.5</td>
<td>-84</td>
</tr>
<tr>
<td>( \alpha )-Ribofuranose</td>
<td>6</td>
<td>105.8</td>
<td>105.8</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Ribofuranose</td>
<td>18</td>
<td>95.2</td>
<td>95.2</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>97.2 ± 2.8</td>
<td>-99.4 ± 13.1</td>
<td></td>
</tr>
</tbody>
</table>

\( \Delta C_p^o/\Delta A_{np} = -1.02 \pm 0.14 \text{ J mol}^{-1} \text{ K}^{-1} \text{ Å}^{-2} \)

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*Percentage of anomeric equilibrium.


Average value weighted with anomeric percentages.


Conformation.
ences, all of which contribute to the binding of Gal[Abe]Man by this antibody. Thus very different interactions may be dominant at different temperatures giving rise to the complex overall temperature dependence. Both van der Waals and hydrogen bonding interactions become weaker with increasing temperature, whereas the hydrophobic effect becomes stronger in terms of free energy. It is perhaps even possible that hydrophilic interactions related to the ability of sugars to bind solvent water molecules in ordered structures via hydrogen bond networks could result in some of the observed change in the heat capacity. Hence, one must be cautious to interpret a large negative $\Delta C_p$ as stemming solely from hydrophilic interactions. It may also be argued that since sugars possess typical amphiphilic surfaces with only relatively small contiguous hydrophobic regions, hydrophobic interactions involving carbohydrates may be of the relatively weak "pairwise" type and not the "bulk-type" interactions characterizing hydrocarbons (46).

van't Hoff Analysis—A van't Hoff plot of the data is shown in Fig. 2. Clearly the plot reveals a biphasic dependence of the binding constant on temperature. Two van’t Hoff enthalpies can be estimated using the following linearized van’t Hoff equation.

$$\ln K = \ln A - \frac{\Delta H_{int}}{RT},$$

where $R$ is the universal gas constant, $\Delta H_{int}$ is the van’t Hoff enthalpy, and $\ln A$ is an intercept related to entropy (the pre-exponential factor). The four leftmost points (high temperatures) yield $\Delta H_{int} = -105.2 \pm 19.7$ kJ mol$^{-1}$ and the three rightmost points (low temperatures) yield $\Delta H_{int} = +105.8 \pm 3.7$ kJ mol$^{-1}$. These values are not approximate averages or even rough estimates of the calorimetrically determined enthalpies in the temperature range, but represent basically the intrinsic binding enthalpies and depend on the choice and definition of the binding constant. Calorimetry measures the total enthalpy including any concomitant reactions that might occur and which might not or only partially influence the binding constant. Any thermodynamic function $\Delta X$ describing the association of the antibody with ligand consists of an intrinsic term and a term referring to possible concomitant reactions, as shown below.

$$\Delta X = \Delta X_{int} + \Delta X_{con}$$

These concomitant reactions can, e.g., be protonation changes, release or uptake of ions, conformational changes, or changes in solvation etc. The calorimetrically observed enthalpy is identical to $\Delta H^0$, i.e. the total enthalpy of all reactions. In contrast to this, the binding constant, which is defined as the ratio between the concentration of the complex and the product of the free antibody and free ligand

$$K = \frac{[A][L]}{[AL]}$$

and which is determined on the basis of the concentration dependence of the evolved heat, basically describes the intrinsic association process and will only reflect any concomitant reactions to the extent that these directly affect $K$ and its temperature dependence. This means that free energies calculated from the following relationship

$$\Delta G^0 = -RT\ln K$$

will be $\Delta G_{con}$ plus the part of $\Delta G_{con}$ that is directly reflected in $K$. Likewise, van’t Hoff enthalpies determined from the temperature dependence of $K$ will represent $\Delta H_{int}$ plus the part of $\Delta H_{con}$ that directly affects $K$ as it is defined (Equation 6). It is not possible from the present data to evaluate the magnitudes of $\Delta G_{con}$ and $\Delta S_{con}$. Nonlinear van’t Hoff plots and differences between van’t Hoff enthalpies and calorimetrically determined enthalpies due to the methodological differences of the two techniques and concomitant reactions are well known in the literature (47–52).

If to a first approximation, $\Delta H_{int}$ is identified with $\Delta H^0$ and $\Delta G^0$ with $\Delta G_{int}$, then apparent values of $\Delta H_{con}$ and $-T\Delta S_{con}$ can be calculated from

$$\Delta H_{con} = \Delta H^0 - \Delta H_{int}$$

and

$$-T\Delta S_{con} = \Delta G^0 - \Delta H_{int}$$

respectively, and these values are summarized in Table III. The transition in the thermodynamics around room temperature is evident. The positive values of $\Delta H_{con}$ above room temperature probably reflect the energy it takes to desoluate the ligand and binding site. It is well established that water molecules form structures around hydrophobic surfaces in such a way that the hydrogen bonds become enthalpically stronger compensated by a loss in entropy (see e.g. Ref. 30).

**Table III**

<table>
<thead>
<tr>
<th>$T$</th>
<th>$\Delta H_{con}$</th>
<th>$\Delta S_{con}$</th>
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</thead>
<tbody>
<tr>
<td>310.06</td>
<td>$-105.2 \pm 19.7$</td>
<td>78 $\pm$ 20</td>
</tr>
<tr>
<td>304.06</td>
<td>$-105.2 \pm 19.7$</td>
<td>77 $\pm$ 20</td>
</tr>
<tr>
<td>298.31</td>
<td>75 $\pm$ 20</td>
<td></td>
</tr>
<tr>
<td>290.83</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>286.10</td>
<td>$-134 \pm 4$</td>
<td></td>
</tr>
<tr>
<td>282.77</td>
<td>$-134 \pm 4$</td>
<td></td>
</tr>
</tbody>
</table>
This has been shown to be true for aqueous solutions of sugars as well in a variety of experiments (38–41, 53, 54). This contrasts with a recent suggestion that ordering of water molecules towards an amphiphilic surface takes place by loss of enthalpy compensated by an entropy gain (8).

The large unfavorable intrinsic enthalpy \(\Delta H_{\text{int}}\) at lower temperatures is largely compensated by a large favorable intrinsic entropy change, but resulting in a net decrease in the magnitude of the free energy. The transition with temperature of the thermodynamics of the association seems to be similar to the phenomenon of cold denaturation of proteins (55, 56), except that the transition temperature appears somewhat higher for the antibody-ligand system than for the thermal stability of most proteins. This destabilization of the complex presumably occurs because the strength of hydrophobic interactions decreases with decreasing temperature.

The entropy \((-T \Delta S^0)\) values in Table I represent the difference between \(\Delta G_{\text{int}}\) and \(\Delta H^0\), i.e. they do not include \(\Delta G_{\text{un}}\). The near constancy of \(\Delta G^0\) clearly shows the compensation between \(\Delta H_{\text{int}}\) and \(-T \Delta S_{\text{int}}\) (Table III), but it is not possible to say whether or not there is a similar compensation between \(\Delta H_{\text{un}}\) and \(-T \Delta S_{\text{un}}\). However, if changes in hydration are the most prominent concomitant reactions, then one might expect a close enthalpy-entropy compensation (slope close to unity). This means that \(\Delta G_{\text{un}}\) will be nearly constant and the compensation plot in Fig. 1 is still valid except for an unknown offset value.

Finally, it must be concluded that it is very difficult to interpret van’t Hoff thermodynamic data as giving information about concomitant reactions such as solvent effects (8), unless it is shown that the van’t Hoff enthalpies do not significantly differ from the calorimetric enthalpies.

**Comparison with the Crystal Structure**—A 2.05Å resolution crystal structure of the Se155–4 Fab complexed with a dodecasaccharide (three repeating units of the O-antigen) was recently published (14). In the crystal, except for the C-6 hydroxyl groups of Man and Gal, all other hydroxyls were found to be involved in hydrogen bonding networks and 15 residues of the complementarity determining regions of the antibody were in van der Waals contacts with the ligand thus creating a tightly fitting binding pocket. Furthermore, 2 tryptophan residues provide stacking interactions with the abequose and galactose units similarly to what has been found for sugar transport proteins (9–10), lysozyme, and phosphorlyase (13). The majority of the amino acid side chains that constitute the binding pocket are aromatic. Thus it is clear from the crystal structure that both hydrophilic as well as hydrophobic interactions are important for binding.

The contact surfaces in the crystal structure of the Se155–4 Fab-dodecasaccharide complex are 304 Å² on the Fab and 255 Å² on the trisaccharide where the abequose contributes 121 Å² (14). The hydrophobic surfaces constitute only a certain fraction of the contact areas. A total hydrophobic contact area of 559 Å² would correspond to an observed heat capacity change of not lower than \(-1000\ J\ mol^{-1}\ K^{-1}\) at 25°C according to the above mentioned relationships, thus indicating that interactions other than hydrophobic ones are important and at least partly responsible for the observed changes in heat capacity.

The importance of the hydrophobic surfaces of carbohydrates and their significance in protein-carbohydrate interactions has been a matter of some controversy in recent years (1, 8, 10). For the system under investigation the binding pocket contains many hydrophobic aromatic side chains (14), but this seems to be a general trend for antibody binding sites where aromatic residues occur with a high frequency (57). The strongest interaction is between the abequose residue (the immunodominant sugar) and the amino acid side chains in the bottom of the pocket. Of course, abequose, being a deoxy sugar, is considerably more hydrophobic than most carbohydrates, and almost all of the side chains interacting with it are aromatic. But the abequose unit also interacts with a structured water molecule through hydrogen bonds with its ring oxygen and its C-4 hydroxyl. It seems reasonable that this particular protein-carbohydrate association is driven to some extent by hydrophobic interactions, but this need not necessarily be the case for protein-carbohydrate interactions in general.

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