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Thermodynamics of Binding of D-Galactose and Deoxy Derivatives thereof to the L-Arabinose-binding Protein

Antonio Hernandez Daranas, Hiroki Shimizu, and Steve W. Homans*

Contribution from the Astbury Centre for Structural Molecular Biology, School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

Received April 5, 2004; E-mail: s.w.homans@leeds.ac.uk

Abstract: We report the thermodynamics of binding of D-galactose and deoxy derivatives thereof to the arabinose binding protein (ABP). The "intrinsic" (solute−solute) free energy of binding ΔG°_{int} at 308 K for the 1-, 2-, 3-, and 6-hydroxyl groups of galactose is remarkably constant (∼−30 kJ/mol), despite the fact that each hydroxyl group subtends different numbers of hydrogen bonds in the complex. The substantially unfavorable enthalpy of binding (∼30 kJ/mol) of 1-deoxygalactose, 2-deoxygalactose, and 3-deoxygalactose in comparison with galactose, cannot be readily accounted for by differences in solvation, suggesting that solute−solute hydrogen bonds are enthalpically significantly more favorable than solute−solvent hydrogen bonds. In contrast, the substantially higher affinity for 2-deoxygalactose in comparison with either 1-deoxygalactose or 3-deoxygalactose derives from differences in the solvation free energies of the free ligands.

Approximately 1% of the human genome codes for proteins involved in glycan synthesis and processing, and much intensive research worldwide is aimed at discovering how glycans modulate physiological processes and the pathogenesis of disease. Such studies are revealing that glycosyltransferases and glycosidases are involved in fundamental processes such as cell−cell communication and signal transduction from cell-surface receptors. However, despite these advances, our knowledge of the molecular basis of carbohydrate-mediated recognition remains severely limited. Indeed, our knowledge of the molecular basis of biomolecular interactions in general is very poor, and there is an urgent need to understand the mechanisms that underly affinity and specificity of such interactions.

A significant number of X-ray crystal structures of carbohydrate−protein complexes can be found in the protein database, and these are unquestionably thought-provoking in terms of the molecular basis of carbohydrate-mediated recognition. However, it is clear from fundamental thermodynamic considerations that both structural and dynamic considerations are of equal importance in governing the affinity of an interaction—the affinity of any given interaction is governed by a complex interplay between the enthalpy and entropy of binding,[1] and hence static X-ray structures only offer partial insight into the binding process. Thus, a number of groups have utilized isothermal titration calorimetry (ITC) to probe the enthalpy and entropy of binding in carbohydrate recognition phenomena,[2−12] and several authoritative reviews exist on the subject.[13−15]

Here, we utilize isothermal titration calorimetry (ITC) combined with high-resolution NMR to study the thermodynamics of glycan−protein interactions, to determine the component enthalpic and entropic contributions to the binding process. The thermodynamics of ligand−protein recognition is enormously complex and involves degrees of freedom in the ligand, protein, complex, and solvent. Hence, to approach the problem in a tractable manner, we have selected a model system, namely the interaction of the arabinose binding protein (ABP) with D-galactose and deoxy derivatives thereof. This system has the advantage that excellent crystal structures are already available for a number of these complexes. While somewhat unique in the high-affinity of carbohydrate binding compared with most other systems, the interactions observed in the crystal structures[16,17] nonetheless indicate that this system is a suitable paradigm.

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Experimental Section

Protein Expression and Purification. The *E. coli* strain overexpressing ABP was a generous gift from Prof. F. Quiocho (Baylor College of Medicine, Houston, Texas). Recombinant ABP was overexpressed at 37 °C in LB Broth and 0.1 mg/mL carbenicillin. Cells were harvested by centrifugation (Sorval RC-5B, SLA-3000, 5000 rpm., 4 °C, 30 min) after an 8 h culture and immediately after the periplasmic space content was selectively extracted using the osmotic shock procedure.[18] Phenylmethanesulfonyl fluoride (PMSF) was added to the osmotic shock fluid to 50 μM. Prior to chromatography, the periplasmic proteins were dialyzed against potassium phosphate buffer (20mM, pH 7.8). Purification of the native protein was achieved in three steps starting with anion exchange chromatography (Pharmacia DEAE Sepharose Fast Flow) using potassium phosphate buffer (20 mM, pH 7.8) and a linear gradient of 0 to 50 mM KCl over two column volumes. Fractions containing ABP were loaded into a chromatofocusing column (Pharmacia PBE 94 Polybuffer Exchanger) using 25 mM imidazole-HCl (pH 7.4) as starting buffer and eluting with a linear gradient of Polybuffer 74-HCl (pH 4.0) over eight column volumes. Finally, gel filtration chromatography (Superdex 200, Pharmacia) using ammonium bicarbonate as mobile phase, yielded ABP >95% pure as estimated by SDS-PAGE. Approximately 20 mg of ABP were obtained per liter of culture. Stable isotopes were incorporated using a medium consisting of 75% Celtone-N (Spectra Stable Isotopes) and 25% of M-9 minimal medium containing ^{15}N-ammonium chloride as nitrogen source.

Isothermal Titration Calorimetry. ITC measurements were performed on a Microcal VP−ITC microcalorimeter which was calibrated using the built-in electrical calibration check. To remove any bound sugar molecules prior to binding studies, the purified protein was dialyzed three times against 1 M guanidine·HCl, 5mM EDTA, 5mM dithiothreitol, 50mM Tris·HCl, pH 8.3; then dialyzed against 5mM dithiothreitol, 5mM EDTA, and 25mM Tris·HCl, pH 8.3 and finally twice against the buffer used for the binding studies, always at 4 °C. Unless stated otherwise, all ITC titrations were conducted in 25 mM Tris·HCl pH 7.4 containing 25 mM EDTA and 1.5 mM NaN_3. Protein concentrations were determined spectrophotometrically from UV absorbance measurements at 280 nm using a molar extinction coefficient $\epsilon_{280} = 64000\ cm^{-1}\ M^{-1}$. Ligand concentrations were determined by ^{1}H NMR using an ethanol internal standard in a single-pulse experiment. Ligand samples were then freeze-dried and redissolved in the same buffer that had been used for protein dialysis. All solutions were degassed immediately prior to use. A typical experiment consisted of a 2 μL injection (discarded in data fitting) followed by 30−45 × 6−8 μL injections of ligand solution into the stirred cell (1.409 mL) containing the protein, using 5 min intervals between injections. For displacement titrations, the protein was preincubated with the low affinity ligand and D-galactose was titrated into the mixture. Data were analyzed using nonlinear least-squares curve fitting in Origin (Microcal Inc.) using the standard one binding site model supplied by Microcal or Sigurskjold's displacement model.[19] This analysis yielded the thermodynamic parameters K (binding constant) and $\Delta H°$ (standard enthalpy of binding). Heats of dilution and mixing were taken into account by subtracting blank titrations of ligand into buffer for direct titrations, but in the case of displacement assays, these effects were accounted for during the fitting process. Heat capacities of binding were obtained from linear least-squares fits to plots of enthalpy versus temperature over the range of 15−35 °C.

NMR spectroscopy. ^{15}N-labeled ABP samples (250 μL) were prepared using 20 mM potassium phosphate buffer (pH 7.0), 3 mM sodium azide, 0.1 mM EDTA, 50 μM protease inhibitor (Pefabloc), and 10% D_2O. NMR experiments were recorded at 308 K on a Varian INOVA 500 spectrometer equipped with triple resonance *z*-gradient probes. The initial protein concentration was 0.3 mM. Aliquots of the sugar solution were added into the NMR tube and vortex-mixed. The final volume increment was less than 10% of the initial volume and the concentration of protein at each point was corrected for dilution from the addition of ligand. A series of seven ^{1}H−^{15}N HSQC spectra, with increasing concentration of sugar, were recorded using the sensitivity enhanced protocol with spectral widths of 8000 and 2200 Hz and 1024 and 128 complex points in the ^{1}H and ^{15}N dimensions, respectively. Data were processed using NMRPipe/NMRDraw[20] using the nlinLS routine to fit peak positions and analyzed using NMRView.[21]

Binding constants were determined by nonlinear least-squares fitting of the chemical shift changes as a function of total ligand concentration using a one site binding model according to the following relationship:

$$\frac{\Delta\delta_i}{\Delta\delta_{max}} = \frac{K_d + [L_T] + [P_T] - \sqrt{(K_d + [L_T] + [P_T])^2 - (4[L_T][P_T])}}{2[P_T]}$$

where $K_d = 1/K_a$ is the dissociation constant, $[L_T]$ and $[P_T]$ are ligand and protein total concentrations, $\Delta\delta_i$ is the chemical shift change after the addition of ligand, and $\Delta\delta_{max}$ is the chemical shift change between the free and the fully bound states. K_d and $\Delta\delta_{max}$ were the fitting parameters in this analysis. When ^{15}N chemical shifts were used, a scaling factor of 0.154 was used. Data fitting was performed with the program Grace 5.1.12.

Cavity Search. X-ray coordinates for ABP complexed with D-Galactose (5abp), D-Fucose (1abf), and L-Arabinose (1abe) were obtained from the Protein Data Bank. Data for ABP in complex with 1-deoxy-D-galactose and 2-deoxy-D-galactose indicate that no extra water molecules are found in those complexes in comparison with D-galactose.[22] However, no such data are available for the 3- and 4-deoxy-D-galactose complexes. Thus, the program PRO_ACT was employed to search for possible bound water locations using a probe radius of 0.95 Å as suggested by the authors.[23] The coordinates for the D-galactose complex (5abp) were used in both cases with the relevant hydroxyl group substituted by a hydrogen atom prior to the cavity search. No extra cavities, suitable to fit a water

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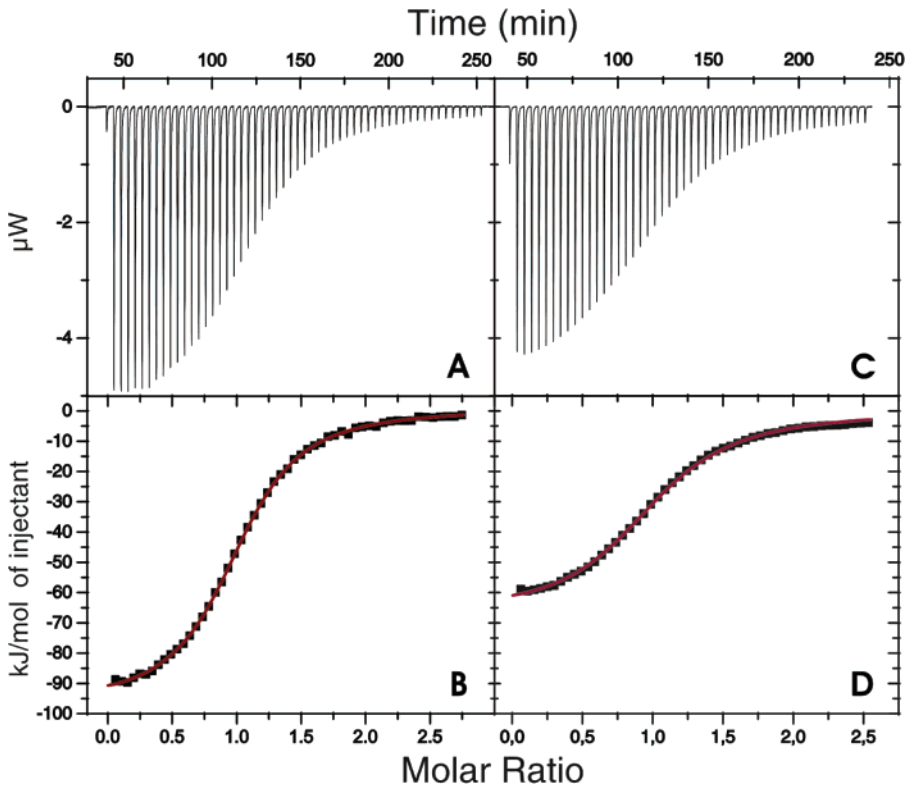

Figure 1. Raw (top) and integrated (bottom) data for titrations of ABP with D-galactose in the absence (A,B) and presence (C,D) of 1-deoxy-D-galactose. The concentrations of protein (30μM) and titrant (0.35mM) were the same in both experiments. Fitted curves based on the one binding site (B) and displacement models (D) are shown in red.

molecule, were found in any monodeoxygalactose derivative. However as a control, the removal of the hydroxymethyl group from D-galactose to transform the ligand into L-arabinose resulted in a complex where a new cavity was found that could accommodate an additional water molecule consistent with the crystal structure of this complex.[16,17]

Chemical Synthesis of Deoxygalactose Derivatives. Full synthetic details for the 1-deoxy-, 3-deoxy-, and 4-deoxygalactose derivatives used in this study can be found in Supporting Information.

Solvation Free Energy Calculations. Solvation standard free energies for galactose and deoxy derivatives were computed with the program AMSOL 7.0,[24] using the AM1 Hamiltonian[25] and a solvent model (AM1-SM4-SRP) parametrized for carbohydrate systems in aqueous solution, as described.[26] All calculations were performed on β-D-configurations with initial geometries defined by the hydroxymethyl group rotamer torsion angle O6−C6−C5−H5 = −60° and hydroxyl group torsion angles (where present) of H1−C1−O1−HO1 = +60°, H2−C2−O2−HO2 = −60°, H3−C3−O3−HO3 = +60°, H4−C4−O4−HO4 = +60°, C5−C6−O6−HO6 = −60°. Trial calculations with alternative low-energy conformations of hydroxymethyl- and hydroxyl-group rotamers, or the α-D anomer, typically gave rise to solvation free energies that differed by about 10% of the absolute free energy of solvation, consistent with the findings of Cramer and Truhlar.[27]

Results and Discussion

Thermodynamics of Binding of Galactose and Derivatives to ABP. The thermodynamics of binding of galactose and each deoxy derivative thereof were determined at 308 K by use of isothermal titration calorimetry. In the case of galactose and 6-deoxygalactose (fucose) "direct" titrations were perfomed, whereas "competition" titrations[19,28] were performed in the case of the remaining deoxy analogues in view of the low affinities. A typical example of the latter type of titration is shown in Figure 1.

The results of these experiments are compiled in Table 1, from which it is clear that the range of affinities spans greater than 4 orders of magnitude.

Similar results were obtained when Tris buffer was substituted by phosphate buffer (data not shown), indicating that there is no contribution to global $\Delta H°$ values from heats of ionization. The affinity for 4-deoxygalactose was too low for thermodynamic parameters of binding to be quantified with any degree of accuracy. In the absence of structural information on this complex, this reduction in affinity is incomprehensible and hence will not be considered further. An early study by Fukada et al.[29] reported K_d = 0.58 mM and $\Delta H°$ = −62.7 kJ/mol for binding of galactose to ABP, which compares favorably with the values reported here taking into account the temperature

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Table 1. Thermodynamics of Binding of D-Galactose and Derivatives to ABP at 308 K

ligand	K_d (μM)	error μM	$\Delta G°$ (kJ/mol)	error	$\Delta H°$ (kJ/mol)	error	$T\Delta S°$ (kJ/mol) (308 K)	error	$\Delta\Delta G°_{solv}$ [a] (kJ/mol)	$\Delta\Delta G°_{int}$ [b] (kJ/mol)	stoichiometry n
galactose	2.2	0.02	−33.36	0.3	−95	0.6	−61	0.6	*c*	−	0.99 ± 0.01
1-deoxy	14600	730	−10.8	0.5	−63	3	−52	3	4.6	27	1.02 ± 0.004
2-deoxy	780	60	−18.3	1.4	−61	4.8	−43	4.8	13.2	28	0.97 ± 0.002
3-deoxy	29620	2620	−9.0	0.8	−57	5	−48	5	8.6	33	0.96 ± 0.005
4-deoxy	>100000	−	−	−	−	−	−	−	nd	−	nd
6-deoxy	25.5	1.6	−27.1	1.7	−93	4	−66	4.3	20.3	27	1.01 ± 0.002

[a] Values reported are the standard free energy of solvation of the respective deoxy analogue minus the standard free energy of solvation of galactose, computed using AMSOL 7.0. [b] $\Delta\Delta G°_{int} = [\Delta G°_{i2} - \Delta G°_{i1}] = [\Delta G°_{obs2} - \Delta G°_{obs1}] + \Delta\Delta G°_{solv}$, where $\Delta\Delta G°_{solv} \approx [\Delta G°_{su2} - \Delta G°_{su1}]$ and the subscripts 1 and 2 refer to galactose and the relevant deoxy analogue, respectively. [c] The absolute solvation free energy of β-D-galactose computed using AMSOL 7.0 is −76.3 kJ/mol.

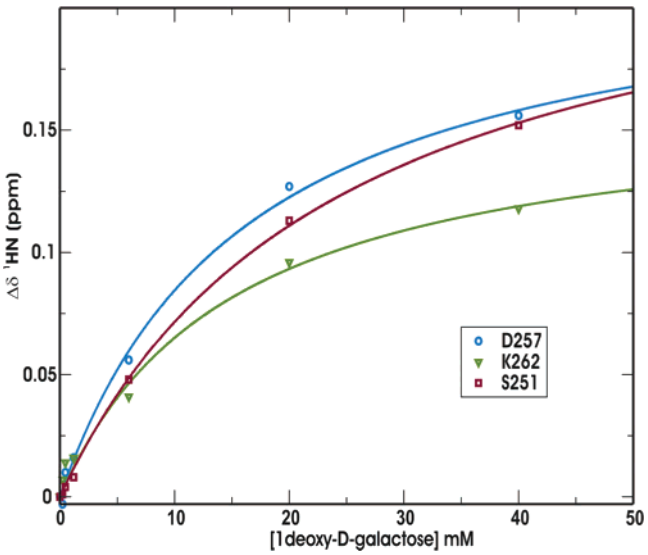
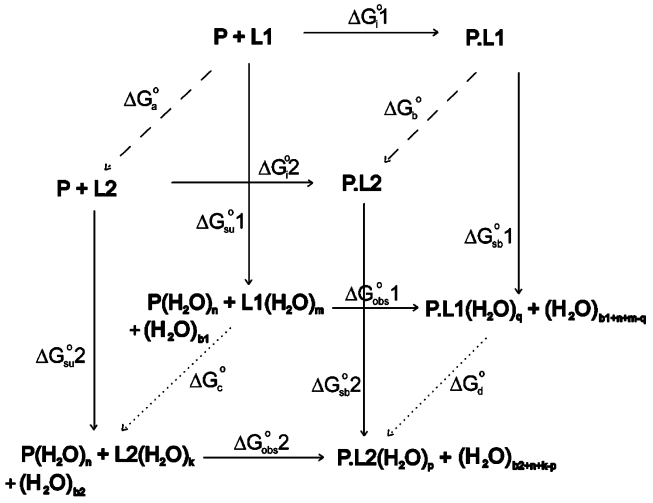


Figure 2. Binding curves resulting from shifts in the H^N backbone resonances of typical binding site residues in ^{15}N-ABP following titration with 1-deoxy-D-galactose. The measured K_d is 17.1 ± 5 mM.

Scheme 1. Born−Haber Cycles for Two Ligands Showing the Relationship between the Observed Free Energy of Binding ΔG_{obs}, the Intrinsic (Solute−Solute) Free Energy of Binding ΔG_i, and the Solvation Free Energies of the Unbound (ΔG_{su}) and Bound (ΔG_{sb}) Species



dependence of the parameters reported in the earlier study. The affinities of galactose, 2-deoxygalactose, and 6-deoxygalactose are approximately 1 order of magnitude lower than those reported by Vermersch et al. using fluorescence titration experiments.[22] The reason for the discrepancy between these data can also be attributed to the differences in temperature between the two data sets (293 K in the previous study versus 308 K in the present study), and the temperature dependences of the affinities for galactose and 6-deoxygalactose (see below) give rise to affinities at 293 K that are essentially in agreement with the data of Vermersch et al. However, the discrepancy of approximately 3 orders of magnitude for 1-deoxygalactose cannot be explained by the higher temperature used in the present study. Therefore, the affinity of 1-deoxygalactose determined in the present study was confirmed by ligand titration using characteristic ^{1}H and ^{15}N NMR chemical shift perturbations of binding-site residues in ^{1}H−^{15}N HSQC spectra of ^{15}N-enriched ABP, using resonance assignments for ABP recently obtained in this laboratory.[30] Typical titration curves are illustrated in Figure 2, and the resulting K_d of 17.1 ± 5 mM is in good agreement with that obtained from ITC measurements (Table 1).

Standard Free Energies of Binding. The measured standard free energies of binding for 1-deoxy-, 2-deoxy- and 3-deoxygalactose are all substantially less favorable than for galactose. Comparison of these values with those for galactose suggests that the standard free energy contribution to binding from a secondary hydroxyl group is between approximately −15 and −24 kJ/mol. This contribution comprises the solute−solute standard free energy of binding plus effects due to the differences in solvation between each ligand, the protein, and each ligand−protein complex (Scheme 1).

The observed difference in binding free energy between two ligands is given by:

$$\Delta G°_{obs2} - \Delta G°_{obs1} = [\Delta G°_{i2} - \Delta G°_{i1}] + \{[\Delta G°_{sb2} - \Delta G°_{sb1}] - [\Delta G°_{su2} - \Delta G°_{su1}]\} \quad (1)$$

The first term on the right-hand side of eq 1 represents the difference in the "intrinsic" (solute−solute) free energy of binding of each ligand L1 and L2 to protein P, whereas the second term represents the difference in solvation free energy, which includes the loss of solute−solvent interactions and the gain in solvent−solvent interactions.[5] In the crystal structures of ABP with galactose, 1-deoxygalactose, and 2-deoxygalactose, there are no significant conformational changes in either the protein or the ligand [22] (apart from the loss of the relevant hydroxyl group). Hence, if the first ligand L1 is identified with galactose and the second ligand L2 is identified with either the 1-deoxy-, 2-deoxy-, or 3-deoxy derivative, then the first term on the right-hand side of eq 1 can be approximately equated

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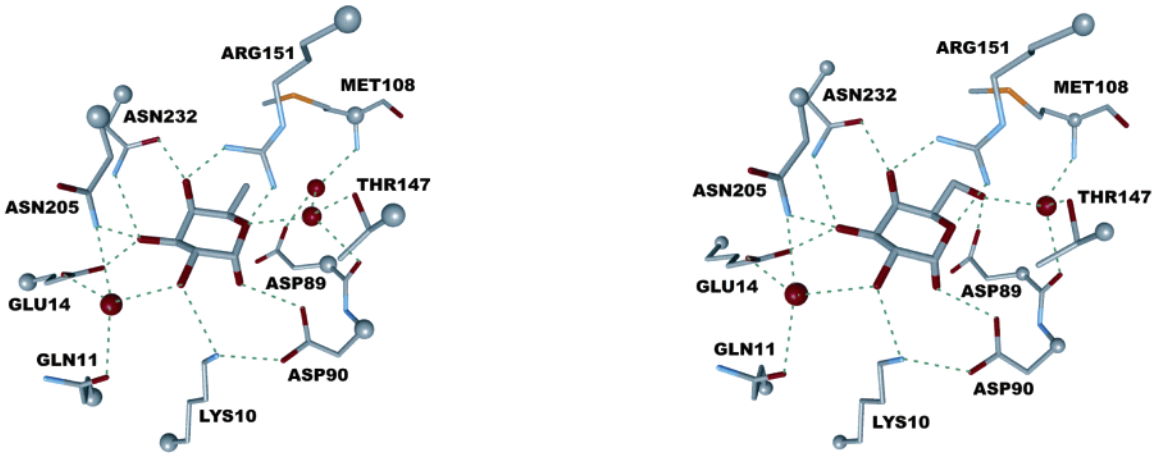

Figure 3. Binding site architecture of the complex of ABP with 6-deoxygalctose (left) and D-galactose (right). [16,17]Bound water molecules are indicated as red spheres.

with the contribution to the solute−solute free energy of binding of secondary hydroxyl group OH1, OH2, or OH3, respectively.

The solvation term is difficult to quantify and cannot readily be accessed experimentally to our knowledge. The complexes of ABP with galactose, 1-deoxygalactose, and 2-deoxygalactose are reported to have the same numbers of bound water molecules;[22] however, structural details of the complex with 3-deoxygalactose have not been reported. However, a cavity search using the program PRO_ACT (see Methods) indicates that additional water molecules are unlikely to be bound in this latter complex. Given the same number and location of bound water molecules in each complex, the solvation term in eq 1 is therefore dominated by the difference in solvation free energy of the free ligands (solvation of the free protein being identical in each case and $[\Delta G^\circ_{sb2} - \Delta G^\circ_{sb1}] \approx 0$). Unfortunately, experimental data on solvation free energies of carbohydrates are not available, principally in view of the fact that their transfer from the water to the vapor phase is effectively undetectable. As an alternative, we therefore sought to compute the solvation free energies for galactose and relevant deoxy derivatives. Initial attempts using free energy perturbation (FEP) calculations[31] were unreliable due to substantial statistical noise derived from the many torsional degrees of freedom in galactose and analogues. As an alternative, we therefore utilized a quantum chemical solvation model based on a continuum treatment of dielectric polarization and solvent-accessible surface area.[24] The advantages and disadvantages of implicit solvation models have recently been reviewed by Cramer and Truhlar.[32] The chosen model has been used successfully to examine the conformational stabilities of glucose in solution.[26,27] In an effort to validate this model for the present application, we determined the solvation free energy difference $\Delta\Delta G^\circ_{solv}$ between two carbohydrate analogues for which approximate experimental solvation free energy data are available, namely tetrahydropyran (thp) and 2-hydroxytetrahydropyran (hthp).[33] The resulting value ($\Delta\Delta G^\circ_{solv} = \Delta G^\circ_{solv(thp)} - \Delta G^\circ_{solv(hthp)} = 10.9$ kJ/mol) is in reasonable agreement with the experimental value of 13.3 kJ/mol, given that the latter is an estimate from a variety of experimental data. Results of AMSOL 7.0 calculations on galactose and relevant deoxy derivatives are given in Table 1. Using the relation $\Delta\Delta G^\circ_{solv} \approx [\Delta G^\circ_{su2} - \Delta G^\circ_{su1}]$, we find that the contribution to the solute−solute standard free energy of binding arising from a single secondary hydroxyl group is remarkably constant at ∼−30 kJ/mol. Thus, the more favorable standard free energy of binding for 2-deoxygalactose in comparison with 1-deoxy- or 3-deoxygalactose derives almost entirely from the significantly more favorable free energies of solvation of the latter compounds (ligand binding being essentially a desolvation process). The constant value of ΔG°_{int} is remarkable given that OH1, OH2, and OH3 support one, two, and three hydrogen bonds, respectively.

In the case of 6-deoxygalactose, the standard free energy of binding is much more favorable than for the other deoxy analogues, and approaches that of galactose. However, the crystal structure of the 6-deoxygalactose−ABP complex reveals that the loss of OH6 is compensated by a second bound water molecule, and one additional hydrogen bond is formed in the 6-deoxygalactose−ABP complex in comparison with the galactose−ABP complex (Figure 3). Moreover, the first bound water molecule is repositioned together with reorientation of certain binding site residue side chains.[34] It is notable that the reduction in binding affinity for 6-deoxygalactose compared with galactose is due largely to a less favorable entropic term (+5 kJ/mol), which compares with an entropic cost between ∼0 and 8 kJ/mol for a single bound water molecule estimated by Dunitz.[35] However, in the complex of ABP with arabinose, where again a second bound water molecule is found in the binding site, we find that the entropic term is 26 kJ/mol less favorable ($\Delta G^\circ = -34.8$ kJ/mol, $\Delta H^\circ = -122$ kJ/mol, $T\Delta S^\circ = -87.2$ kJ/mol). Thus, the interpretation of the binding thermodynamics of 6-deoxygalactose is not straightforward due to a number of competing factors.

Temperature Dependence of Thermodynamic Parameters. In an attempt to obtain further insight into the entropies of binding, the temperature dependence of the thermodynamics of the binding of galactose and 6-deoxygalactose was also investigated using ITC, and the results are listed in Table 2. The magnitude of ΔH° is a function of the heat capacity at constant pressure, ΔC_p, which can be determined directly from the

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Table 2. Temperature Dependence of the Binding of D-Galactose and 6-Deoxy-D-galactose to ABP

ligand	K_d (μM)	error	$\Delta G°$ (kJ/mol)	error	$\Delta H°$ (kJ/mol)	error	$T\Delta S°$ (kJ/mol)	error
				308 K				
galactose	2.2	0.02	−33.36	0.02	−95.0	0.6	−61.6	0.6
6-deoxy	25.5	1.6	−27.1	0.2	−93	4	−66	4.3
				298 K				
galactose	0.61	0.02	−35.45	0.1	−87.4	0.84	−52.0	0.84
6-deoxy	6.67	0.09	−29.53	0.03	−81.67	0.5	−52.1	0.45
				288 K				
galactose	0.52	0.04	−34.63	0.19	−81.8	1.34	−47.2	1.3
6-deoxy	2.43	0.07	−30.95	0.08	−74.43	0.93	−43.5	0.9

temperature dependence of $\Delta H°$. A simple linear model provides estimates of ΔC_p for the binding of galactose and 6-deoxygalactose of -656 ± 57 and -936 ± 124 J/mol/K, respectively. Since the early work of Kauzmann, [36] large changes in heat capacity have been identified as a signature for hydrophobic interactions.[37] A significant negative ΔC_p of binding suggests burial of hydrophobic surface area, based on the good correlation between ΔC_p and changes in surface area in a number of systems.[38] The larger negative ΔC_p for the more hydrophobic 6-deoxygalactose in comparison with that for galactose is consistent with this interpretation. By use of solvent-accessible surface areas for galactose and 6-deoxygalactose computed from AMSOL 7.0 calculations, together with eq 12 of ref 35, $\Delta\Delta C°_P$ is estimated to be ~ -90 J/mol/K. In addition, the contribution to $\Delta\Delta C°_P$ arising from the sequestration of one additional water molecule in the complex with 6-deoxygalactose can be estimated as ~ -75 J/mol/K,[12,37] giving a total $\Delta\Delta C°_P = -165$ J/mol/K. This result is in satisfactory agreement with the experimental value of $\Delta\Delta C°_P = -280 \pm 136$ J/mol/K.

Overall Thermodynamics of Binding. Binding to ABP of all ligands described in the present study is enthalpy driven. With the exception of charged ligands such as heparin and heparin sulfate, both $\Delta H°$ and particularly $T\Delta S°$ are significantly larger than typical values reported for the vast majority of carbohydrate−protein interactions,[13] including oligosaccharides. The reason for these anomalously large values cannot be delineated with certainty from global thermodynamics measurements derived from ITC. It is noteworthy, however, that the $\Delta H°$ value for binding of galactose compared with that for 1-deoxy-, 2-deoxy-, or 3-deoxygalactose is favorable by $\sim$30 kJ/mol. Naively, one might interpret this additional enthalpic contribution to binding in the case of galactose as arising from the additional hydrogen bond(s) that form due to the additional hydroxyl group in the complex. However, prior to binding, the ligand is hydrogen bonded to solvent water, the enthalpic contribution of which is contained within $\Delta\Delta G^o_{solv}$. Intuitively, one would anticipate that the enthalpic component ($\Delta\Delta H^o_{solv}$) for solvation of deoxy analogues of galactose compared with that for galactose is endothermic, i.e., solvation of deoxy analogues is enthalpically less favorable than that of galactose. If one assumes momentarily that the enthalpic contribution from ligand−protein hydrogen bonds is effectively zero, on the basis that hydrogen bonds to solvent exist prior to the association, one must conclude that the enthalpies of solvation of the 1-deoxy, 2-deoxy-, and 3-deoxy analogues of galactose are each more favorable than that of galactose by ~ -30 kJ/mol, which is counterintuitive. While the unfavorable entropic contribution arising from the loss of a given hydroxyl group may comprise a number of factors, including the influence of an unpaired polar side chain, van der Waals interactions, or nonpolar interactions during complex formation, the conclusion that ligand−protein hydrogen bonds are enthalpically significantly more favorable than ligand−solvent hydrogen bonds would appear to be inescapable, at least in this system. This is in marked contrast to the conclusions of Connelly et al. in the study of FK506 binding interactions.[39] The large negative $T\Delta S°$ of binding might in turn arise from significant restriction of protein degrees of freedom due to the strong nonbonded interactions with the ligand. Alternatively, as suggested by Lemieux,[40] the unfavorable $T\Delta S°$ term might derive from disordered water molecules adjacent to polyamphiphilic "hydraphobic" surfaces which are less structured in comparison with bulk water. It should be possible to distinguish between these two possibilities by measurement of $T\Delta S°$ for protein degrees of freedom on a per-residue basis by use of NMR relaxation experiments.[41−43] Resonance assignments in ABP have recently been completed,[30] and such measurements are in progress.

Conclusions

In this study we have compared the thermodynamics of binding of galactose and various deoxy derivatives thereof to the arabinose binding protein. A combination of isothermal titration calorimetry experiments together with calculation of relative ligand solvation free energies, reveals that the contribution to the solute−solute free energy of binding from the OH1, OH2, OH3, and OH6 groups appears to be remarkably constant at ~ -30 kJ/mol, despite the fact that each hydroxyl group subtends different numbers of hydrogen bonds in the complex. The substantially unfavorable enthalpy of binding ($\sim$30 kJ/mol) of 1-deoxygalactose, 2-deoxygalactose, and 3-deoxygalactose in comparison with that of galactose, cannot be readily accounted for by differences in ligand solvation, suggesting that ligand−protein hydrogen bonds are enthalpically significantly more favorable than ligand−solvent hydrogen bonds. The significant affinity of ABP for 6-deoxygalactose, which is only $\sim$1 order of magnitude weaker than galactose, derives in part from the incorporation of an additional water molecule that substitutes for OH6. In contrast, the substantially higher affinity for 2-deoxygalactose in comparison with that for either 1-deoxygalactose or 3-deoxygalactose derives not from differences in solvation of the binding site but from differences in the solvation free energies of the free ligands. We have recently noted similar thermodynamics governing the specificity of binding of pyrazine derivatives to the major urinary protein,[44] suggesting that the modulation of binding specificity by ligand solvation thermodynamics may be a general phenomenon.

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Supporting Information Available: Details of the synthesis of 1-deoxy-, 3-deoxy- and 4-deoxy-D-galactopyranose. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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