Thermodynamics of α-Cyclodextrin−*p*-Nitrophenyl Glycoside Complexes. A Simple System To Understand the Energetics of Carbohydrate Interactions in Water

Elena Junquera,[†,‡] José Laynez,[§] Margarita Menéndez,[§] Sunil Sharma,[†] and Soledad Penadés*[,†]

Grupo de Carbohidratos, Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006-Madrid, Spain, and Instituto de Química Física Rocasolano, CSIC, Serrano 119, 28006-Madrid, Spain

Received April 9, 1996[⊗]

Thermodynamic studies of the binding of a series of *p*-nitrophenyl glycosides (PNPGly) of varying stereochemistry to α-cyclodextrin (α-CD) were performed at three different temperatures (25, 35, and 42 °C) using a microcalorimetric technique. The system *p*-nitrophenol (PNP) at pH = 3 and α-CD was also studied for the sake of comparison. All these complexes were found to be enthalpy driven with a favorable enthalpic term clearly dominant over an unfavorable entropic term. A clear enthalpy−entropy compensation effect was observed at all the temperatures, with a slope close to unity (α = 1.02) and an intercept $T\Delta S°_o = 2.91$ kcal mol^{-1}. This thermodynamic pattern is in agreement with those usually found for lectin−carbohydrate associations and for the binding processes of several host−guest systems. This pattern is explained in terms of the contribution of primarily two driving forces: the van der Waals interactions between the host and the guest, and the solvation/desolvation processes which accompany the association reaction. The presence of the carbohydrate molecule in the PNP ring causes a slight destabilization of the complex at 25 °C with respect to the α-CD−PNP (pH = 3) complex, although a different behavior has been observed depending on the axial/equatorial configuration of the glycoside and the temperature. This behavior is modulated by the stereochemistry of the glycoside. Differences were observed between the deoxy-derivatives (LAra and LFuc) and those derivatives with a hydroxymethyl group (Glc, Gal, Man). $\Delta C_p°$ values were obtained from the dependency of $\Delta H°$ on temperature ($=(\partial\Delta H°/\partial T)_p$). These values are small and negative except for αMan complex. For the latter complex, discrepancy between the calorimetric and the calculated van't Hoff enthalpies was observed. Parallels are drawn between the thermodynamics of our model and those proposed for carbohydrate−protein associations.

Introduction

Molecular recognition of carbohydrates is an important and controversial topic. Structural studies with lectins and antibodies,[1] and more recently with glycosphingolipids,[2] have given information about the forces involved in this process. Hydrogen bonding interactions with polar groups of the protein, van der Waals contacts with nonpolar protein side chains, participation of divalent cations (Ca^{2+}, Mg^{2+}) in ternary complexes, and water molecules mediating hydrogen bonding interactions are common carbohydrate binding motifs. However, structural information alone is not sufficient to establish the role played by these polar and nonpolar forces in the selectivity and stability of association. Accurate values of enthalpy ($\Delta H°$), entropy ($\Delta S°$), and heat capacity ($\Delta C_p°$) changes are neccesary to understand how these intermolecular forces contribute to the overall free energy ($\Delta G°$) of complexation.

Recently, calorimetric studies have allowed the proposal of models for the energetics of carbohydrate−protein association.[3] From these studies, some trends emerge. All associations are enthalpy driven and they show a remarkable linear relationship between the decrease in enthalpy and the compensating decrease in entropy. Moreover, the $\Delta C_p°$ values obtained to date, indicate differences in the energetics of lectin−carbohydrate and antibody−carbohydrate binding.[3] Lectin−carbohydrate associations are usually characterized by small and negative $\Delta C_p°$ values,[4] while antibody−carbohydrate $\Delta C_p°$ values are large and temperature

* To whom correspondence should be addressed.
† Instituto de Química Orgánica.
‡ From Departamento de Química Física I, Universidad Complutense de Madrid, 28040-Madrid, Spain, in a postdoctoral stay.
§ Instituto de Química Física Rocasolano.
⊗ Abstract published in *Advance ACS Abstracts*, September 1, 1996.

(1) (a) Lavie, A.; Allen, K. N.; Petsko, G. A.; Ringe, D. *Biochemistry* **1994**, *33*, 5469−5480. (b) Vyas, M. N.; Vyas, N. K.; Quiocho, F. A. *Biochemistry* **1994**, *33*, 4762−4768. (c) Beierbeck, H.; Delbaere, L. T. J.; Vandonselaar, M.; Lemieux, R. U. *Can. J. Chem.* **1994**, *72*, 463−470. (d) Delbaere, L. T. J.; Vandonselaar, M.; Prasad, L.; Quail, J. W.; Wilson, K. S.; Danter, Z. *J. Mol. Biol.* **1993**, *230*, 950−965. (e) Rini, J. M.; Hardman, K. D.; Einspahr, H.; Suddath, F. L.; Carver, J. *J. Biol. Chem.* **1993**, *268*, 10126−10132. (f) Lobsanov, Y. D.; Gitt, M. A.; Leffler, H.; Barondes, S. H.; Rini, J. M. *J. Biol. Chem.* **1993**, *268*, 27034−27038. (g) Wright, C. S.; Jaeger, J. *J. Mol. Biol.* **1993,** *232*, 620−638. (h) Sharff, A. J.; Rodseth, L. E.; Quiocho, F. A. *Biochemistry* **1993**, *32*, 10553−10559. (i) Cygler, M.; Rose, D. R.; Bundle, D. R. *Science* **1991**, *253*, 442−445. (j) Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Science* **1988**, *242*, 1290−1295.

(2) (a) Hakomori, S. *Pure. Appl. Chem.* **1991**, *63*, 473−482. (b) Kojima, N.; Hakomori, S. *Glycobiology* **1991**, *1*, 623−630. (c) Misevic, G. N.; Burger, M. M. *J. Biol. Chem.* **1993**, *268*, 4922−4929. (d) Stewart, R. J.; Boggs, J. M. *Biochemistry* **1993**, *32*, 10666−10674. (e) Dammer, U.; Popescu, O.; Wagner, P.; Angelmetti, D.; Güntherodt, H.-J.; Misevic, G. N. *Science* **1995**, *267*, 1173−1175.

(3) For an interesting discussion about the energetic contributions on protein−carbohydrate interactions see: Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719−728 and references therein.

(4) (a) Chevernak, M. C.; Toone, E. J. *Biochemistry* **1995**, *34*, 5685−5695. (b) Mandal, K. D.; Kishore, U.; Fred Brewer, C. *Biochemistry* **1994**, *33*, 1149−1156. (c) Schwarz, F. P.; Puri, K. D.; Bhat, R. G.; Surolio, A. *J. Biol. Chem.* **1993**, *268*, 7668−7677. (d) Williams, B. A.; Chevernak, M. C.; Toone, E. J. *J. Biol. Chem.* **1992**, *267*, 22907−22911. (e) Bains, G.; Lee, R. T.; Lee, Y. C.; Freire, E. *Biochemistry* **1992**, *31*, 12624−12628. (f) Schwarz, F. P.; Puri, K.; Surolia, A. *J. Biol. Chem.* **1991**, *266*, 24344−24350.

S0022-3263(96)00659-7 CCC: $12.00

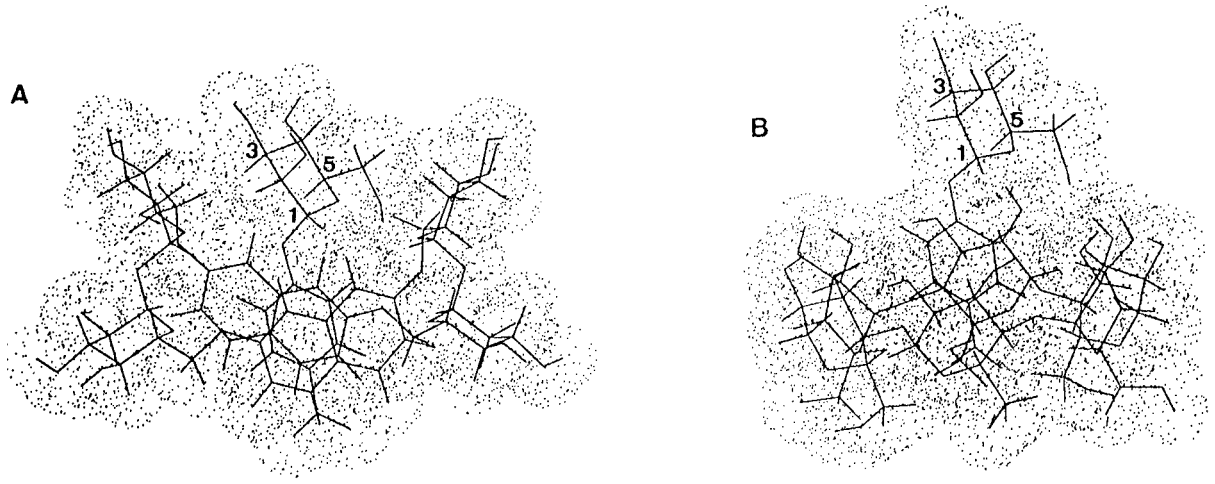


Figure 1. Comparison of the calculated geometries of the inclusion complexes between the *p*-nitrophenyl α-D-mannopyranoside and (A) a glycophane and (B) α-cyclodextrin.

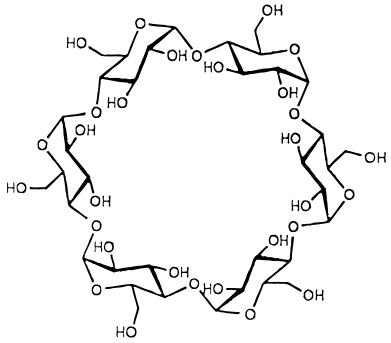
dependent.[5] These differences have been interpreted in terms of a great participation of hydrophobic interactions and solvent effects during binding in carbohydrate−antibody associations as compared to carbohydrate−lectins associations. The molecular mechanism which drives these complexations is as yet not well understood, and fundamental questions related to the contributions of atomic interactions and hydration effects to the enthalpy and free energy of binding have to be answered. The favorable contribution of hydrogen bonds versus desolvation of lipophilic surfaces to the enthalpy of binding is a subject of discussion.[6] The origin of entropy changes, which oppose binding, is interpreted by some authors in terms of ligand/protein decreased flexibility upon binding,[7] while other authors attribute this compensation to the unique nature of water.[8] Also, enhanced solute−solute interactions offset by a more unfavorable configurational entropy of binding has been suggested to be the origin of enthalpy−entropy compensation in lectin−carbohydrate associations.[4a]

Since the thermodynamics of host−guest complexation in aqueous solutions is closely related to complexation in biological systems, molecular recognition studies with model receptors can help to understand the fundamental driving forces involved in the energetics of biological interactions. Among the many possible model receptors, cyclodextrins (CD's)[9] and cyclophanes (CP's)[10] have been proven to be excellent models for studying the nature of the apolar binding of neutral organic molecules in aqueous solutions. Calorimetric studies with cyclophanes[11] and cyclodextrins[12] have shown the enthalpy-driven character of these processes and that a large part of the favorable enthalpy of binding must result from specific contributions made by the solvent.[11a]

We have recently used model systems to evaluate and understand the interaction of carbohydrates in water.[13] These systems are constituted by either α-cyclodextrin (α-CD) or a glycophane (a cyclodextrin−cyclophane hybrid receptor made up of α,α trehalose and naphthalene molecules) as receptors and a series of *p*-nitrophenyl glycosides (PNPGly) as substrates. The free energy of binding ($\Delta G°$) for this interaction, determined by ^{1}H NMR spectroscopy, was in the range of −2.6 to −3.3 kcal mol^{-1}. In the glycophane + PNPGly system, a stabilizing contribution up to 1.8 kcal mol^{-1} to $\Delta G°$ due to the carbohydrate moieties was observed, while this stabilization was not present in the α-CD−PNPGly complexes.[13] We have attributed these differences in complexing properties to the different geometry of both complexes, which makes van der Waals contacts between lipophilic carbohydrate surfaces of both glycophane and PNPGly possible, while in the α-CD complexes the carbohydrate moiety of the guests remains mainly in contact with the bulk water (Figure 1). This distinct orientation of the carbohydrate during binding makes both complexes adequate models to evaluate the influence, on the energetics of binding, of carbohydrate residues that interact by lipophilic forces with the receptor (Figure 1A), and

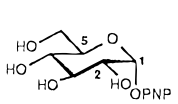
(5) Sigurskjold, B. W.; Bundle, D. R. *J. Biol. Chem.* **1992**, *267*, 8371−8376.
(6) (a) Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347−374. (b) Quiocho, F. A. *Pure Appl. Chem.* **1989**, *61*, 1293−1306.
(7) Carver, J. P. *Pure Appl. Chem.* **1993**, *65*, 763−770.
(8) (a) Lumry, R.; Rajender, S. *Biopolymers* **1970**, *9*, 1125−1227. (b) Lemieux, R. U.; Delbarre, L. T. J.; Beierbeck, H.; Spohr, U. In *Host-Guest Molecular Interactions: From Chemistry to Biology* (Ciba Foundation Symposium 158); Chadwick, D. J.; Widdows, K., Eds.; Wiley: Chichester, 1991; pp 231−248.
(9) (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978. (b) Bergeron, R. J. In *Inclusion Compounds;* Academic Press: New York, 1984; Vol. 3, Chapter 12. (c) Cramer, F.; Saenger, W.; Spatz, H. Ch. *J. Am. Chem. Soc.* **1967**, *89*, 14−20. (d) Breslow, R. *Advances in Enzymology*; A. Meisler: New York, 1986; pp 1−60.
(10) Diederich, F. *Cyclophanes*; Stoddart, J. F., Ed.; Royal Society of Chemistry: Cambridge, 1991.
(11) (a) Smithrud, D. B.; Wyman, T. B.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5420−5426. (b) Diederich, F.; Smithrud, D. B.; Sanford, E. M.; Wyman, T. B.; Ferguson, S. B.; Carcanagne, D. R.; Chao, I.; Houk, K. N. *Acta Chem. Scand.* **1992**, *46*, 205−215. (c) Stauffer, D. A.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Org. Chem.* **1990**, *55*, 2762−2767.
(12) (a) Eftink, M. R.; Harrison, J. C. *Bioorg. Chem.* **1981**, *10*, 388−398. (b) Harrison, J. C.; Eftink, M. R. *Biopolymers* **1982**, *21*, 1153−1166. (c) Eftink, M. R.; Andy, M. L.; Byström, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765−6772. (d) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475−481. (e) Inoue, Y.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 10637−10644. (f) Rekharsky, M. V.; Schwarz, F. P.; Tewari, Y. B.; Goldberg, R. N.; Tanaka, M.; Yamashoji, Y. *J. Phys. Chem.* **1994**, *98*, 4098−4103. (g) Rekharsky, M. V.; Schwarz, F. P.; Tewari, Y. B.; Goldberg, R. N. *J. Phys. Chem.* **1994**, *98*, 10282−10288. (h) Namor, A. F. D. *Pure Appl. Chem.* **1993**, *65*, 193−202. (i) Hirsch, W.; Muller, T.; Pizer, R.; Ricatto, P. J. *Can. J. Chem.* **1995**, *73*, 12−15. (j) Bertrand, G. L.; Faulkner, J. R.; Han, S. M.; Armstrong, D. W. *J. Phys. Chem.* **1989**, *93*, 6863−6867. (k) Qi, H.; Nishihata, T.; Rytting, J. H. *Pharm. Res.* **1994**, *11*, 1207−1210. (l) Rekharsky, M. V.; Goldberg, R. N.; Schwarz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Am. Chem.* **1995**, *117*, 8830−8840.
(13) (a) Coterón, J. M.; Vicent, C.; Bosso, C.; Penadés, S. *J. Am. Chem. Soc.* **1993**, *115*, 10066−10076. (b) Jiménez-Barbero, J.; Junquera, E.; Martín-Pastor, M.; Sharma, S.; Vicent, C.; Penadés, S. *J. Am. Chem. Soc.* **1995**, *117*, 11198−11204.

Chart 1

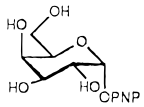


Axial glycosides

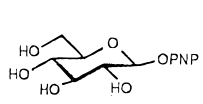
Equatorial glycosides



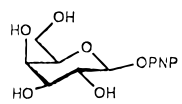
α-Glc



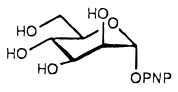
α-Gal



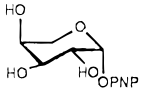
β-Glc



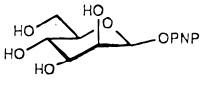
β-Gal



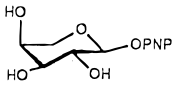
α-Man



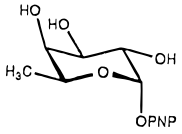
β-L-Ara



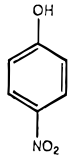
β-Man



α-L-Ara

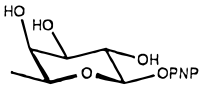


α-L-Fuc



PNP

(pH=3)



β-L-Fuc

those residues that are exposed mainly to the bulk water[14] (Figure 1B).

We now present our initial calorimetric study of the interaction between α-cyclodextrin and a series of *p*-nitrophenyl glycopyranosides (PNPGly) of D-glucose (Glc), D-galactose (Gal), D-mannose (Man), L-arabinose (LAra), and L-fucose (LFuc), with axial and equatorial configuration at the anomeric center (Chart 1). These substrates have been chosen because of their distinct configuration which allowed us to evaluate both the influences of stereochemistry and the chemical nature of the substituents on the thermodynamics of binding. The system formed by *p*-nitrophenol (PNP) at pH = 3 and α-CD has also been studied to analyze the effect of the presence of the carbohydrate moiety in the association. The study has been carried out at three different temperatures (25, 35, and 42 °C) in order to have a complete thermodynamic picture of the studied interaction. By having accurate values for $\Delta G°$, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ of this system, we expected to gain insight into (a) the contribution of the different forces to the energy of binding (van der Waals interactions, hydrophobic effect, hydrogen-bonding, solvent reorganization), (b) the role played by the hydroxyl groups on the interaction and their contribution to the enthalpy and entropy of binding, (c) the influence of stereochemistry of these hydroxyl groups on the energetics of binding, and (d) the role of water in these processes. We had also expected to be able, from the comparison of $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ values of both α-CD and the glycophane systems, to interpret the origin of the additional stabilization found in the glycophane system, due to the presence of lipophilic carbohydrate−carbohydrate interactions.[13] Unfortunately, the low solubility of the glycophane in water did not allow until now a calorimetric study with this model system.

Experimental Section

Materials. α-Cyclodextrin, of >99% purity, was purchased from Fluka. PNP and the PNPGly were purchased from Sigma. The water content of α-CD, as determined by a Karl-Fischer analysis, was 10% mass, and this hydrated molecular weight was used when preparing solutions. Both α-CD and PNPGly were used without further purification, while the PNP was recrystallized twice from chloroform. A glycine/HCl buffer solution was prepared for the calorimetric titration of PNP at pH = 3.

Methods. A LKB batch microcalorimeter equipped with a 2107-350 LKB titration unit was used for the measurement of both the association constants and the standard molar enthalpy changes for the binding processes of the PNPGly and/or the PNP (pH = 3) to αCD at three different temperatures, 25, 35, and 42 °C. A computer controlled the injections and

(14) (a) Lemieux, R. U.; Du, M.-H.; Spohr, U. *J. Am. Chem. Soc.* **1994**, *116*, 9803−9804. (b) Du, M.-H.; Spohr, U.; Lemieux, R. U. *Glycoconjugate J.* **1994**, *11*, 443−461.

collected the titration data. The thermodynamic parameters K and $\Delta H°$ for all the studied systems were obtained from the titration curves by using a program made by us in TURBO C language, which fits the experimental data with a nonlinear least squares method based on a Marquardt algorithm. The principle of the measurement, the technical description, and the detailed experimental procedures were reported previously.[15]

The calorimeter was calibrated both electrically and chemically at each operating temperature. In a typical run, a α-CD solution at its solubility limit (around 0.1 M) was injected into the reaction vessel where 2 mL of the PNPGly solution was placed at a concentration around 2×10^{-3} M. Approximately, 16 injections (15.6 μL per injection) of the α-CD solution were made in every titration experiment. Thus, the concentration of the α-CD solution varied from 0.77 mM to 11 mM. In order to ensure a high degree of complexation at the titration end point, and considering the small association constants in these kinds of systems, two saturation points were measured separately in the batch mode of the calorimeter by mixing the reactants at saturating concentrations of α-CD with respect to PNPGly concentration.

Whenever the calorimetric experiments were carried out using the titration unit, the ligand dilution heat was canceled by use of the reference cell, and the small differential compression and mixing heat effects were determined separately and substracted from the heat quantities evolved in the reaction experiments. In those experiments performed without the titration system, the dilution heat of both α-CD and PNPGly was measured in a separate run and was found to be negligible.

Most complexation enthalpies and association constants reported in this work were obtained in duplicate or triplicate runs. Their uncertainties, given by the nonlinear least squares analysis, are less than 3% and 15% for $\Delta H°$ and the binding constants, respectively.

Results and Discussion

Cyclodextrins (CD's) and guest molecules (G) are known to form inclusion complexes of mainly 1:1 stoichiometry, following the equilibrium:

$$CD + G \rightleftharpoons CD:G \quad (1)$$

where the equilibrium or association constant is given by the expression:

$$K = [CD:G]/([CD][G]) \quad (2)$$

Since the substances studied herein are nonelectrolytes, their activity coefficients will be close to unity at the low concentrations used in these experiments, and expression 2 is given with concentrations instead of activities. Therefore, nonideality corrections are assumed to be negligible for both the measured equilibrium constants and the standard molar association enthalpies. Calorimetric titrations have been performed at 25, 35, and 42 °C for the systems formed by α-CD and the *p*-nitrophenyl glycosides (PNPGly) of D-glucose (Glc), D-galactose (Gal), D-mannose (Man), L-arabinose (LAra), and L-fucose (LFuc), with axial and equatorial configuration at the anomeric center, as well as *p*-nitrophenol (PNP, pH = 3). The titration calorimetric curves for the α-CD + α- and β-D-Glc-PNP systems at 25 °C are represented as a function of α-CD concentration in Figure 2, where the solid lines represent the fitting calculated values, assuming a 1:1 stoichiometry. The thermodynamic parameter values, $\Delta H°$, $T\Delta S°$, $\Delta G°$, and K, together with their corresponding uncertainties, are reported in Table 1 at the three temperatures; these data are graphically visualized in Figure 3 at 25 °C.

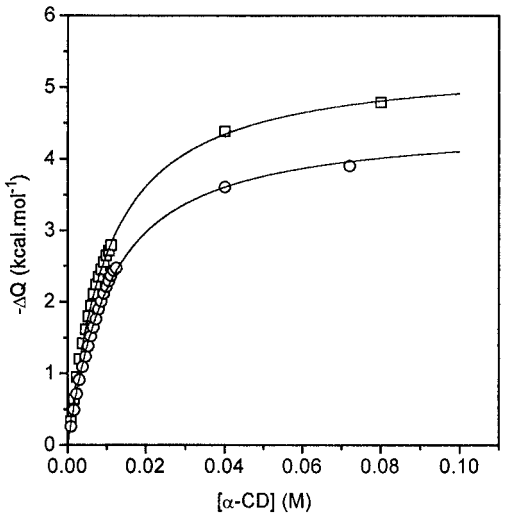


Figure 2. Experimental heats of complexation at 25 °C as a function of α-CD concentration for the systems constituted by α-CD and: □, *p*-nitrophenyl α-D-glucopyranoside; ○, *p*-nitrophenyl β-D-glucopyranoside. The solid lines represent the calculated values.

The free energies of binding, $\Delta G°$, of PNPGly with α-CD were also determined by ^{1}H NMR spectroscopy[13] and the values obtained agree with those obtained here by calorimetry. The induced chemical shifts observed upon inclusion of all the guests helped us also to establish an approximate geometry for all the complexes. The upfield shifts observed in the H3 (~0.24 ppm) and H5 (~0.03 ppm) protons of α-CD, located inside the cavity, indicate the inclusion of the guest aromatic ring. Moreover, the downfield shifts observed for the ortho (~0.18 ppm) and meta (~0.25 ppm) aromatic protons also confirm this inclusion. Additionally, the larger downfield shift induced in the meta with respect to that in the ortho aromatic protons, together with the larger upfield shift observed for the H3 proton with respect to the H5 proton of α-CD, suggests an inclusion geometry through the wider rim of α-CD as represented in Figure 4.[16,17]

The values in Table 1 and Figure 3 reveal some points for discussion: (i) the complex formation is exothermic and enthalpy driven, (ii) the presence of the carbohydrate guest moiety has a different effect on the thermodynamic parameters depending on temperature and sugar configuration, (iii) $\Delta C_p°$ values decrease from Man > Glc ≥ Gal ≥ Ara ≅ Fuc ≅ PNP (pH = 3) for both axial and equatorial glycosides, and (iv) the enthalpy and the entropy of binding are always compensated irrespective of the temperature.

Enthalpic Driving Force. As can be seen in Table 1 and in Figure 3, all the complexes formed by PNPGly or PNP and α-CD are exothermic and enthalpy driven near room temperature, each with an enthalpic term

(15) Chen, A.; Wadsö, I. *J. Biochem. Biophys. Methods* **1982**, *6*, 307−316.

(16) The proposed geometries are based on preliminary calculations using a Silicon Graphics workstation with the MM3* force field as integrated in MACROMODEL v. 4.5. The atomic coordinates of the crystal structure of α-CD[17] were used to obtain a conformational minimum for the receptor. Every substrate, previously minimized,[13] was docked manually into the cavity by both faces of the receptor and then minimized. The steric energies for docking the substrates into the narrow rim of α-CD were higher than those for docking into the wider rim.

(17) Hingerty, B.; Saenger, W. *J. Am. Chem. Soc.* **1976**, *98*, 3357−3365.

Table 1. Thermodynamics of Binding of PNP-Glycosides or PNP (pH = 3) to α-Cyclodextrin at 25, 35, and 42 °C

PNPGly	K (M^{-1})	$-\Delta G°$ (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})
		Axial Glycosides		
		T = 25 °C		
αGlc	111 ± 14	2.79 ± 0.03	5.4 ± 0.1	−2.6 ± 0.1
αGal	94 ± 12	2.69 ± 0.04	5.0 ± 0.1	−2.3 ± 0.1
αMan	140 ± 18	2.93 ± 0.03	5.8 ± 0.1	−2.9 ± 0.1
βLAra	146 ± 19	2.95 ± 0.03	5.7 ± 0.1	−2.8 ± 0.1
αLFuc	126 ± 15	2.87 ± 0.04	5.1 ± 0.1	−2.2 ± 0.1
PNP	192 ± 25	3.11 ± 0.03	6.0 ± 0.1	−2.9 ± 0.1
		T = 35 °C		
αGlc	115 ± 15	2.91 ± 0.04	5.3 ± 0.1	−2.4 ± 0.1
αGal	90 ± 12	2.76 ± 0.03	5.3 ± 0.1	−2.5 ± 0.2
αMan	138 ± 18	3.02 ± 0.03	5.2 ± 0.1	−2.2 ± 0.1
βLAra	99 ± 12	2.81 ± 0.03	6.6 ± 0.2	−3.8 ± 0.2
αLFuc	108 ± 12	2.87 ± 0.05	5.6 ± 0.2	−2.7 ± 0.1
PNP	104 ± 10	2.84 ± 0.05	7.3 ± 0.2	−4.5 ± 0.2
		T = 42 °C		
αGlc	104 ± 14	2.91 ± 0.05	5.3 ± 0.1	−2.4 ± 0.2
αGal	73 ± 9	2.69 ± 0.02	5.4 ± 0.1	−2.7 ± 0.2
αMan	142 ± 18	3.10 ± 0.05	5.0 ± 0.1	−1.9 ± 0.2
βLAra	78 ± 9	2.73 ± 0.02	7.3 ± 0.2	−4.6 ± 0.2
αLFuc	73 ± 9	2.69 ± 0.02	6.4 ± 0.2	−3.7 ± 0.2
PNP	82 ± 11	2.76 ± 0.02	7.5 ± 0.2	−4.7 ± 0.2
		Equatorial Glycosides		
		T = 25 °C		
βGlc	104 ± 13	2.75 ± 0.04	4.5 ± 0.1	−1.8 ± 0.1
βGal	121 ± 16	2.84 ± 0.04	4.1 ± 0.1	−1.3 ± 0.1
βMan	113 ± 15	2.80 ± 0.04	4.8 ± 0.1	−2.0 ± 0.1
αLAra	80 ± 10	2.60 ± 0.04	5.2 ± 0.1	−2.6 ± 0.1
βLFuc	109 ± 13	2.78 ± 0.04	4.6 ± 0.1	−1.8 ± 0.1
PNP	192 ± 25	3.11 ± 0.03	6.0 ± 0.1	−2.9 ± 0.1
		T = 35 °C		
βGlc	110 ± 14	2.88 ± 0.05	4.6 ± 0.1	−1.7 ± 0.2
βGal	120 ± 16	2.93 ± 0.04	4.2 ± 0.1	−1.3 ± 0.1
βMan	77 ± 10	2.66 ± 0.04	4.9 ± 0.1	−2.2 ± 0.1
αLAra	79 ± 9	2.68 ± 0.04	5.8 ± 0.1	−3.1 ± 0.1
βLFuc	78 ± 9	2.67 ± 0.03	5.4 ± 0.1	−2.7 ± 0.1
PNP	104 ± 10	2.84 ± 0.05	7.3 ± 0.2	−4.5 ± 0.2
		T = 42 °C		
βGlc	88 ± 11	2.80 ± 0.05	5.1 ± 0.1	−2.3 ± 0.2
βGal	78 ± 10	2.73 ± 0.05	4.8 ± 0.1	−2.1 ± 0.2
βMan	85 ± 11	2.78 ± 0.05	5.1 ± 0.1	−2.3 ± 0.2
αLAra	62 ± 7	2.58 ± 0.02	6.3 ± 0.2	−3.7 ± 0.2
βLFuc	60 ± 7	2.56 ± 0.02	5.7 ± 0.1	−3.1 ± 0.2
PNP	82 ± 11	2.76 ± 0.02	7.5 ± 0.2	−4.7 ± 0.2

clearly favorable ($\Delta H° < 0$) and an unfavorable entropic term ($T\Delta S° < 0$). This behavior responds to the thermodynamic pattern usually found for monosaccharides binding to lectins[3] and also for the association between small guests molecules and an apolar cavity in water[11,12c−g,l] where the enthalpy of binding is more negative than the free energy of binding. However, this is not what is to be expected for a typical hydrophobically driven process, such as the formation of micelles and membranes[18] where positive change in entropy and close to zero enthalpy change are observed. A combination of hydrophobic effect ($\Delta H°$ around 0; $\Delta S°$ positive), van der Waals forces ($\Delta H°$ negative; $\Delta S°$ negative), and solvent reorganization could account for the apparent thermodynamic parameters of PNP and PNPGly binding to α-CD. As long as the formation of the complexes becomes more exothermic, the complexation entropy becomes less favorable, irrespective of temperature and guest configuration. This correlation between the enthalpic and the entropic terms is shown in Figure 5, where $T\Delta S°$ is plotted as a function of $\Delta H°$ at 25, 35, and 42 °C. The

(18) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; Wiley: New York, 1980.

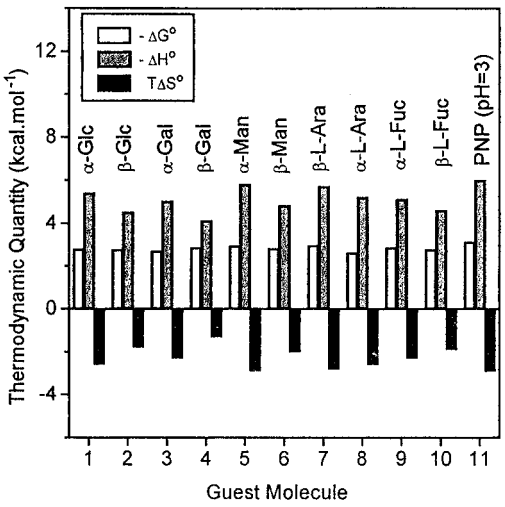


Figure 3. Free standard energy ($-\Delta G°$), standard enthalpy ($-\Delta H°$) and standard entropy terms ($T\Delta S°$) for the inclusion of *p*-nitrophenyl glycosides (PNPGly) and *p*-nitrophenol (PNP) into the α-CD cavity in aqueous solution at 25 °C.

slope (α = 1.02), the intercept ($T\Delta S°_o$ = 2.91 kcal mol^{-1}), and the linear correlation coefficient (r = 0.9987) of the compensation plot agree with those obtained for other kinds of 1:1 host−guest associations.[11,12d−g,j,l] Slopes of α = 1.1 and intercepts of 4.4 kcal mol^{-1} have been found for lectin−carbohydrate interactions,[3,4] while values of 0.9 and 5.8 kcal mol^{-1} were found for antibody−carbohydrate associations,[5] respectively.

Effect of the Carbohydrate Moiety of the PNP-Glycosides. As can be observed in Table 1, the presence of a monosaccharide in the *p*-nitrophenol molecule results in formation of inclusion complexes with lower or slightly higher stability than that of the PNP complex, depending on temperature and nature of the sugar moiety. Thus, at 25 °C the stability of both axial and equatorial PNPGly complexes is always lower than that of PNP complexes, while at 35 and 42 °C is similar or slightly higher for axial PNPGly complexes, while equatorial PNPGly give equally or slightly less stable complexes than the PNP at the same temperature. These results indicate that the presence of the carbohydrate residue on the PNP does not always result in a lower binding to the receptor. This is in contrast to the additional stabilization of about 1 kcal mol^{-1} provided by the carbohydrate moiety in the glycophane−glycoside complexes.[13] The effect of the carbohydrate on $\Delta G°$ is slightly lower for the axial than for the equatorial glycosides at the three temperatures studied. Due to the $\Delta H°/\Delta S°$ compensation effect on $\Delta G°$, the influence of the stereochemistry of the sugar on complex stability is more evident in the enthalpic and entropic terms. Within the temperature range studied, variations in stability ($\delta\Delta G°$) for the axial PNPGly are due, on average, to a decrease of about 10−22% in the enthalpic term ($\delta\Delta H° > 0$) and to a reduction of around 12−37% in the entropy loss ($\delta\Delta S° > 0$), with respect to the values found for the complex α-CD−PNP. In the case of equatorial PNPGly, a 20−30% decrease in the negative enthalpic term and a clear decrease in the entropy loss ($\delta\Delta S° > 0$) ranging from 35 to 47% is observed within the same temperature range. These data indicate that the enthalpy change of binding is less favorable for both axial and equatorial glycosides than for the PNP complex, whereas the entropy change is less unfavorable for the PNPGly complexes.

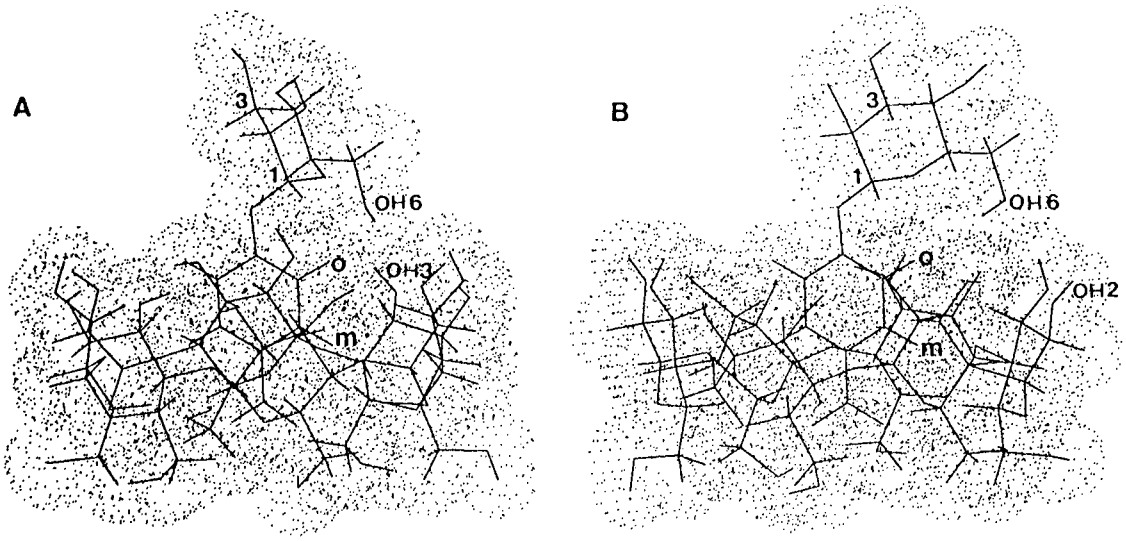


Figure 4. Preliminary molecular modeling simulation of the inclusion complexes between α-CD and the axial (A) and equatorial (B) *p*-nitrophenyl D-mannopyranosides.

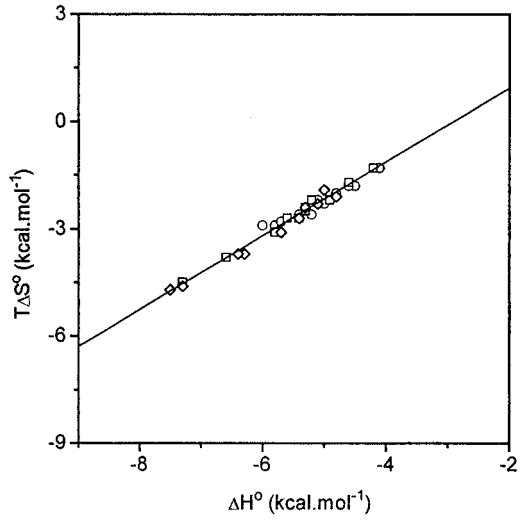


Figure 5. Enthalpy−entropy compensation plot for the inclusion of PNPGly and PNP into the α-CD cavity in aqueous solution at: ○, 25 °C; □, 35 °C, and ◇, 42 °C.

Effect of the Stereochemistry of the PNP-Glycoside on the Thermodynamics of the Inclusion Process. It is evident from data in Table 1 that inclusion complexes formed by α-CD and the axial PNPGly are slightly more stable, at the three temperatures measured, than those with equatorial configuration, with the only exception of the α-CD + α/*β*-Gal-PNP system. At 25 °C, the negative enthalpy change is more favorable (around 1 kcal mol^{-1} for Glc, Gal, and Man, and approximately 0.5 kcal mol^{-1} for LAra and LFuc) for the axial than for the equatorial glycosides. The entropy loss is also higher (around 1 kcal mol^{-1} for Glc, Gal, and Man and approximately 0.3 kcal mol^{-1} for LAra and LFuc, in terms of $T\Delta S°$) for the axial than for the equatorial partner. A similar trend, within the experimental incertitude, is also observed at higher temperatures. This feature is evident in the experimental curves in Figure 2, where the saturation curve for the axial glycoside runs over the one for the equatorial glycoside. The fact that the entropy change was less unfavorable for the equatorial than for the axial glycosides, with the exception of the manno derivative, could be explained taking into account that the solvated equatorial glycosides present a more rigid conformation in aqueous solution, due to the exo-anomeric effect,[19] than their axial partners do. On the other hand, the higher exothermicity observed for axial-complex formation suggests that axial disposition of the aromatic ring favors the inclusion of these glycosides into the α-CD cavity over the equatorial one. This conclusion is also supported by the ^{1}H NMR data obtained at 30 °C. At similar glycoside:α-CD concentration ratios (∼1:35), a higher induced chemical shift is observed for the meta aromatic protons in the axial glycosides (∼0.24 ppm) than in the equatorial (∼0.15 ppm), suggesting a stronger binding and/or a deeper inclusion of the axial glycosides in the cavity (Figure 4). This effect may be due to the relatively different orientation of the sugar and aromatic rings in the axial and equatorial glycosides.

The chemical nature of the substituents in the sugar moieties seems also to influence the binding. From the computer-generated geometries for axial and equatorial complexes in Figure 4, it is to be expected that changes at C1, C2, and C5 of the guest, near the CD cavity entrance, will have more influence on the binding parameters than changes at C3 and C4, further away from the cavity and in contact with the bulk water. In fact, significant variations on the thermodynamic parameters are only observed by modifying position 5 in the glycosides. At 25 °C, the substitution of an equatorial CH_2-OH group in αGal for an equatorial H (αGal → *β*LAra), increases the enthalpy gain in 0.7 kcal mol^{-1} in the axial glycoside, while in the equatorial (*β*Gal → αLAra) a higher increase ($\delta\Delta H° = 1.1$ kcal mol^{-1}) is obtained. The result is an additional stabilization of 0.26 kcal mol^{-1} at 25 °C and a negligible effect at 35 °C and 42 °C for the axial glycosides (αGal → *β*LAra), while in the equatorial (*β*Gal → αLAra) a destabilization is observed at all temperatures. A similar trend is found by substitution of the hydroxyl group at position 6 in the galacto derivative for hydrogen (DGal → LFuc). In this case, smaller values of $\delta\Delta H°$ and $\delta(T\Delta S°)$ are observed. Thus, the presence of an apolar group (hydrogen or methyl) at C5 slightly stabilizes the binding in the axial glycosides, while in the equatorial a comparable destabilizing effect is observed. In the proposed geometries for both axial

(19) Lemieux, R. U.; Koto, S.; Vorsin, D. *Anomeric Effect. Origin and Consequences. ACS Symp. Ser.* **1979**, *87*, 17−29.

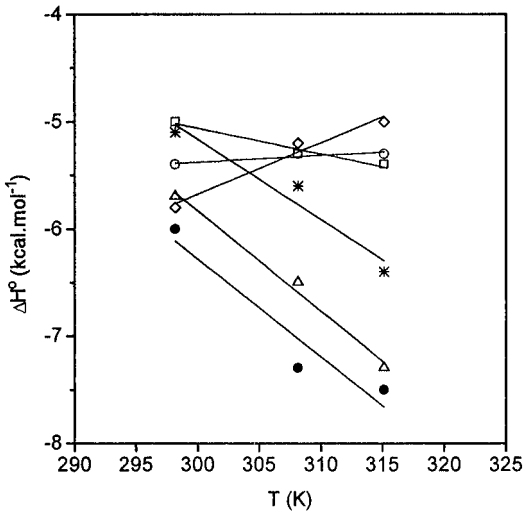


Figure 6. Standard enthalpy ($\Delta H°$) of association as a function of temperature for the systems constituted by α-CD and: ♢, αMan-PNP; ○, αGlc-PNP; □, αGal-PNP; *, αLFuc-PNP; △, βLAra-PNP; and ●, PNP in aqueous solution.

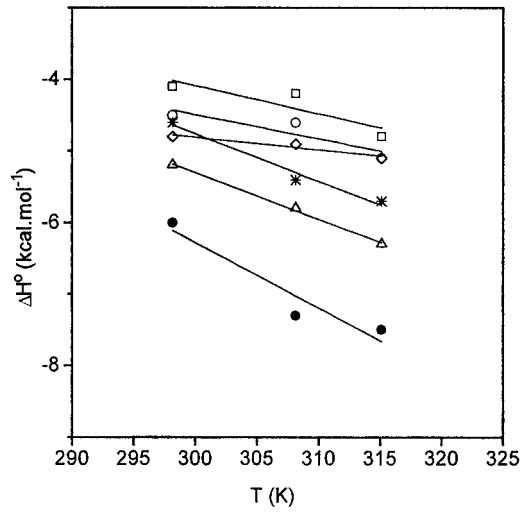


Figure 7. Standard enthalpy ($\Delta H°$) of association as a function of temperature for the systems constituted by α-CD and: ♢, βMan-PNP; ○, βGlc-PNP; □, βGal-PNP; *, βLFuc-PNP; △, αLAra-PNP; and ●, PNP in aqueous solution.

and equatorial complexes (Figure 4), the hydroxyl group at C6 in the PNPGly has an adequate distance (~3 Å) to interact by way of hydrogen bonding with the OH2 and OH3 groups of α-CD. However, this hydroxyl group does not seem to form productive H-bonding interactions with α-CD, because its substitution by hydrogen results in slightly more favorable $\Delta H°$ of binding. The development of more favorable van der Waals interactions between both host and guest carbohydrate surfaces, together with solvent reorganization,[20] may be the origin of the observed increase in $\Delta H°$ on going from galacto to arabino and fuco configurations. The introduction of a methyl group at position 5 (LAra → LFuc) results in positive $\delta\Delta H°$ and $\delta(T\Delta S°)$, reflecting unfavorable van der Waals interactions but favorable hydrophobic effects.

The effects on the thermodynamic parameters, by changing from equatorial to axial the hydroxyl group at position 2 (DGlc → DMan) and 4 (DGlc → DGal, although very small, may indicate that the orientation of hydroxyl groups in the sugar moiety, even though they are apart from the interacting center, could have an influence on the energetics of binding. Similar effects have been found in biological systems. Surolia et al.[4c] have observed that the association with three different lectins is weakened on going from Man to Glc in spite of the noninvolvement of the OH2 group in direct interactions with the proteins. Changes in the $\Delta G°$ of oligosaccharide−protein binding, that result from alterations in the chemical nature of a substituent which comes near but not neccesary in contact with the receptor, have been attributted to changes in hydration.[14a]

Temperature Dependence. As can be observed in Table 1, a different behavior according to the temperature is observed for the axial and equatorial glycosides in their associations with α-CD. The free energy of binding, $\Delta G°$, is almost constant with the temperature, although small changes depending on the stereochemistry of the sugar moiety are observed. However, differences are observed in the variation of $\Delta H°$ with temperature, as can be seen in Figures 6 and 7. In the equatorial glycosides (Figure 7), the standard enthalpy of binding $\Delta H°$ becomes more exothermic as temperature increases, in the order PNP > αLAra ≅ βLFuc > βGal > βGlc > βMan, while in the axial glycosides (Figure 6) this trend is only observed for βLAra, αLFuc, and αGal. The enthalpy of binding is constant or even less favorable with increasing temperature for the αGlc and αMan derivatives, respectively. The $T\Delta S°$ values vary with temperature in the opposite direction, but to a greater extent than $\Delta H°$ values. These results indicate a different behavior for the *p*-nitrophenyl α-mannopyranoside, with respect to the other glycosides. The values of the standard molar heat capacity changes $\Delta C_p°$ $(=(\partial\Delta H°/\partial T)_p)$ calculated from the temperature dependency of $\Delta H°$ (see Figures 6 and 7) are shown in Table 2. The PNP binds to α-CD with a $\Delta C_p°$ of −91 cal mol^{-1} K^{-1}, while the $\Delta C_p°$ values for the glycosides are small and negative, and range from −17 to −93 cal mol^{-1} K^{-1} with the exception of αMan derivative. Negative $\Delta C_p°$ values are usually found for the inclusion of apolar solutes by cyclodextrins[12b,f] and cyclophanes[11] and for carbohydrate association with lectins[3,4] in aqueous solutions. Nevertheless, positive and temperature dependent values of $\Delta C_p°$ have been found for the binding of oligosaccharides to monoclonal antibodies.[5] The positive

Table 2. Heat Capacity ($\Delta C_p°$), Average Calorimetric Enthalpy ($\Delta H_{cal}°$), and van't Hoff Enthalpy ($\Delta H_{VH}°$) Changes for the Binding of PNPGly and PNP to α-Cyclodextrin

PNPGly	$\Delta C_p°$ [a]	$\Delta C_p°_{30}$ [b]	$\Delta C_p°_{38.5}$ [b]	$-\Delta H_{cal}°$ [c]	$-\Delta H_{VH}°$ [c]	$-\Delta H_{cal}°/-\Delta H_{VH}°$
			Axial Glycosides			
αGlc	6	10	0	5.3	0.6	8.8
αGal	−24	−30	−14	5.2	2.6	2.0
αMan	48	60	28	5.3	−0.1	53.0
βLAra	−93	−90	−100	6.5	6.9	0.9
αLFuc	−75	−40	−115	5.7	5.7	1.0
PNP	−91	−130	−28	6.9	9.4	0.7
			Equatorial Glycosides			
βGlc	−34	−10	−70	4.7	1.6	2.9
βGal	−40	−10	−85	4.4	4.4	1.0
βMan	−17	−10	−28	4.9	3.4	1.4
αLAra	−64	−60	−75	5.8	2.6	2.2
βLFuc	−66	−80	−43	5.2	6.5	0.8
PNP	−91	−130	−28	6.9	9.4	0.7

[a] Calculated from a linear regression analysis, in cal mol^{-1} K^{-1}. [b] Estimated as $\Delta\Delta H°/\Delta T$, in cal mol^{-1} K^{-1}. [c] In kcal mol^{-1}.

(20) (a) Chevernak, M. C.; Toone, E. J. *J. Am. Chem. Soc.* **1994**, *116*, 10533−10539. (b) Connelly, P. R.; Thomson, J. A.; Fitzgibbon, M. J.; Bruzzese, F. J. *Biochemistry* **1993**, *32*, 5583−5590.

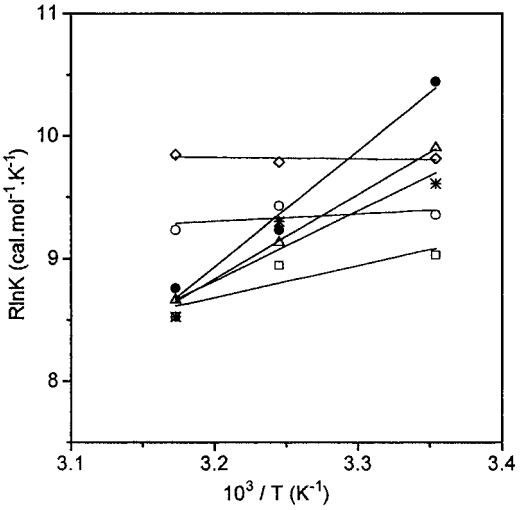


Figure 8. Van't Hoff plots for the systems constituted by α-CD and: ◇, αMan-PNP; ○, αGlc-PNP; □, αGal-PNP; *, αLFuc-PNP; △, *β*LAra-PNP; and ●, PNP in aqueous solution.

$\Delta C_p°$ obtained for the αMan complex seems to be unusual.[3] However, a detailed examination of the scarce calorimetric data for carbohydrate−lectin association also shows small positive $\Delta C_p°$ values for the binding of the monosaccharides MeαMan (+14 cal mol^{-1} K^{-1}), Man (+116 cal mol^{-1} K^{-1}) and MeαGal (+25 cal mol^{-1} K^{-1}) to concavalin A, pea lectin,[4c] and the winged beam lectin,[4f] respectively. Positive $\Delta C_p°$ values can result from hydrophilic interactions[5] related to the ability of sugars to bind solvent water molecules in ordered structures.[21] Small $\Delta C_p°$ values may also be associated to small conformational change upon binding.[4e] Although the $\Delta C_p°$ values here found are calculated from only three temperatures, examination of Table 2 suggests a certain temperature dependence. Similar dependence has been also observed by Diederich et al.[11a] in cyclophane systems. Bundle et al.[5] have also found an even stronger *T* dependence for $\Delta C_p°$ in carbohydrate−antibody associations. In both cases, strong discrepancies between the calorimetric determined enthalpies and the enthalpies calculated from van't Hoff analysis were found.

Comparison of the van't Hoff enthalpy values ($\Delta H_{VH}°$), calculated from the van't Hoff plot slopes (Figure 8) and the average calorimetric values ($\Delta H_{cal}°$) indicates differences between $\Delta H_{cal}°$ and $\Delta H_{VH}°$ for αGlc and αMan derivatives (Table 2). The latter discrepancy cannot be due to experimental errors, since the PNP and the deoxysugar as well as the galacto, gluco, and manno derivatives have all been studied under the same experimental and reproducible conditions. Discrepancies between calorimetric and van't Hoff enthalpy values have been found in many natural and artificial systems.[5,11a,12f,22] The origin of these discrepancies has been attributed to concomitant processes such as protonation, hydration, or conformational changes which accompany the binding reactions, so that the binding constant defined by eq 2 is an apparent equilibrium constant, whose dependency with temperature may differ from the experimentally determined enthalpy change.[23] In our system, a possible conformational change associated with the binding are that related to the gg:gt:tg rotameric equilibrium involving the hydroxymethyl group at C5 of the gluco, galacto, or manno derivatives. This equilibrium depends on the stereochemistry of the sugar and on solvent effects;[24] the solvent water seems to lower the barrier between the conformers.[25] In fact, in the gluco and manno configuration the gg and gt are the preferred conformers (~60:40) in water, while in galacto configuration the favored equilibrium is gt:tg (~60:40).

Other possible source of concomitant enthalpy could be due to changes in hydration of both ligands and binding site upon association. Guests that interact strongly with their solvent cage are supposed to show large negative $\Delta C_p°$ values. The small $\Delta C_p°$ values for Glc, Gal, and Man, which change from negative to positive in the order αGal → αGlc → αMan, could indicate that the manno derivative is the sugar that least interacts with its solvent cage. In a systematic study of a series of monosaccharides in aqueous solution, Galema et al.[26] proposed a stereospecific hydration model which classifies the carbohydrates into three groups depending on the stereochemistry of the hydroxyl groups at positions 2 and 4. The thermodynamic parameters sensitive to the stereochemistry in the hydration process, i.e. the apparent partial molar isentropic compressibilities κ_{s12} and hydration numbers n_h, were found to decrease in the order Man > Glc > Gal, which agrees with the tendency found by us for $\Delta C_p°$ values. It is worthy to note that in our results $\Delta C_p°$ values follow the same trend independent of the axial or equatorial configuration at the anomeric center, becoming more negative in the order Man > Glc > Gal > LFuc ~ LAra ~ PNP (see Figures 6 and 7). Additionally, the magnitude of the discrepancies found between $\Delta H_{cal}°$ and $\Delta H_{VH}°$ increases in the order αGal < αGlc < αMan. These results may indicate that the variation of the thermodynamic inclusion parameters with the stereochemistry of the glycoside here observed, may be due in part to an intrinsic behavior of the carbohydrate molecule involving both the conformational equilibrium of the hydroxymethyl group and/or the specific arrangement of the water molecules around its amphiphilic surfaces. Furthermore, these results support the idea of considering hydration effects as important contributions to the energetics of biological associations.[4d,8,20,27,28] Recently, it has also been shown that solvent reorganization provides a favorable contribution to the neat enthalpy of binding in lectin−

(21) (a) Frank, F. *Pure Appl. Chem.* **1987**, *59*, 1189−1202. (b) Brady, J. W.; Schmidt, R. K. *J. Phys. Chem.* **1993**, *97*, 958−966.

(22) (a) Naghiti, H.; Tamura, A.; Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5597−5599. (b) Sirgurskjold, B. W.; Berland, C. R.; Svensson, B. *Biochemistry* **1994**, *33*, 10191−10199. (c) Lin, Y.; Sturtevant, J. M. *Protein Sci.* **1995**, *4*, 2559−2561.

(23) Eftink, M. R.; Biltonen, R. *Thermodynamic of Interacting Biological Systems* in *Biological Microcalorimetry;* Beezer, A. E., Ed.; Academic Press: London, 1980; pp 343−412.

(24) (a) Bock, K.; Duus J. Ø. *J. Carbohydr. Chem.* **1994**, *13*, 513−543. (b) Cramer C. J.; Truhlar D. G. *J. Am. Chem. Soc.* **1993**, *115*, 5745−5753. (c) Kroon-Batenburg L. M. J.; Kroon, J. *Biopolymers* **1990**, *29*, 1243−1248.

(25) Zuccarello, F.; Buemi, G. *Carbohydr. Res.* **1995**, *273*, 129−145.

(26) Galema, S. A.; Høiland, H. *J. Phys. Chem.* **1991**, *95*, 5321−5326.

(27) Bundle, D. R.; Eichler, V.; Gidney, M. A. J.; Meldal, M.; Raganskas, A.; Sigurskjold, B. W.; Sinnot, B.; Watson, D. C.; Yaguchi, M.; Martin Young, N. *Biochemistry* **1994,** *33*, 5172−5182.

(28) For important contributions about the role of water in biological associations see: (a) Rand, R. P. *Science* **1992**, *256*, 618. (b) Ernst, J. A.; Clubb, R. T.; Zhon, H.-X.; Gronenborn, A. M.; Clore, G. M. *Science* **1995**, *267*, 1813−1817. (c) Rand, R. P.; Fuller, N. L.; Butko, P.; Francis, G.; Nicholls, P. *Biochemistry* **1993**, *32*, 5925−5929. (d) Robinson, C. R.; Sligar, S. G. *J. Mol. Biol.* **1993**, *234*, 302−306. (e) Colombo, M. F.; Rau, D. C.; Parsegian, V. A. *Science* **1992**, *256*, 655−659. (f) Honing, B.; Nicholls, A. *Science* **1995**, *268*, 1144−1149. (g) Wolfenden, R.; Radzicka, A. *Science* **1994**, *265*, 936−937.

carbohydrate associations, $\Delta C_p°$ being a measure of this solvent reorganization.[20]

Conclusions

This paper constitutes the first systematic study on the thermodynamics of carbohydrate associations in water with an artificial receptor. It has been shown that our simple α-CD + PNPGly system can give insight in the energetics of carbohydrate interactions in water. The results presented in this paper call for some remarks. First, the thermodynamic values obtained with our simple system are close in magnitude to those obtained for the binding of monosaccharides to proteins.[3,4,27] That is noteworthy taken into account that the only structural homology between the α-CD + PNPGly system and the biological systems is the carbohydrate moiety and the solvent water. Similar consideration has been already pointed out by Toone et al.[4d] Second, the complex formation is enthalpy driven near room temperature, with enthalpy−entropy compensation. Both hydration effects and van der Waals interactions are proposed to be the main driving forces for this association process. Although the proposed geometries of the complexes indicate the possibility of hydrogen bonding interactions between the OH2 and OH3 groups of the receptor and the OH6 of the sugar residues, functional group replacement shows that they do not contribute significantly to the free energy of binding. Third, the enthalpy of binding is provided mainly by the PNP moiety; the stereochemistry of the carbohydrate moiety, however, modulates the thermodynamics of binding. Fourth, in contrast with the large negative $\Delta C_p°$ values found for many biochemical reactions,[29] small and negative $\Delta C_p°$ values were obtained for all the systems, with the exception of αMan derivative. The different behavior of the latter compound can be explained in terms of different hydrophilic interactions related to the ability of sugars to structure water molecules in a stereospecific way. This effect, together with possible changes in the gg:gt:tg rotameric conformational equilibrium of the hydroxymethyl group at C5, could also explain the origin of the discrepancies found between the $\Delta H_{cal}°$ and $\Delta H_{VH}°$ for these glycosides.

Finally, what this picture seems to reveal is that the chemical nature and the stereochemistry of the substituents in the carbohydrate has a differential effect on the thermodynamics of binding in aqueous solution, even though they are mainly exposed to the solvent water. In these cases, specific interactions with the receptor are expected to be minimal, and it may be that the differences in the thermodynamic parameters, in fact, reflect an intrinsic variation in the state of the solvated free substrates[30] upon binding. It would be of interest to carry out systematic thermodynamic studies on the specific hydration of oligosaccharides, in general, and of *p*-nitrophenyl glycosides, in particular, to clarify this point. Comparative studies on the energetics of binding in light and heavy water of our simple system could give insight into the contribution of hydration to the enthalpy of binding.

Acknowledgment. This work was supported by the Dirección General de Investigación Científica y Técnica (Grant PB93-0127). We thank Prof. Martín-Lomas for his continous support. S.K.S. thanks the Ministerio de Educación y Ciencia for a postdoctoral fellowship. We also thank M. Martín-Pastor for performing the computer-generated representation of the complexes α-CD: *p*-nitrophenyl mannopyranosides.

Supporting Information Available: Three figures related to the NMR results (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO960659G

(29) Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2236−2240.

(30) For similar consideration in organic solvents see: Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271.