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Kinetics of Binding of Methyl α- and β-D-Galactopyranoside to Peanut Agglutinin: A Carbon-13 Nuclear Magnetic Resonance Study†

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ABSTRACT: The binding kinetics of methyl α- and methyl β-D-galactopyranoside to the anti-T lectin from peanuts were studied by ^{13}C NMR, employing methyl galactopyranosides specifically enriched in ^{13}C at C-1. Association and dissociation rate constants, as well as their activation parameters, are reported. The association rate constants, 4.6×10^4 M^{-1} s^{-1} for the α-galactopyranoside and 3.6×10^4 M^{-1} s^{-1} for the β-galactopyranoside, are several orders of magnitude below those expected for a diffusion-controlled process. For both anomers, the association rate constant was temperature independent, implying that the association process occurs without a significant activation enthalpy. However, a considerable association activation entropy was found for both ligands. The dissociation rate constants were in the range of 9–46 s^{-1} within a temperature range of 5–35 °C for the α-galactopyranoside, and in the range of 9–39 s^{-1} within a temperature range of 5–25 °C for the β-galactopyranoside. A considerable dissociation activation enthalpy of ca. 10 kcal mol^{-1} was found for both anomers. A two-step binding model, consistent with the present NMR data and with previous UV and CD spectroscopic data, is presented.

Considerable information regarding the specificities and dimensions of lectin–carbohydrate binding sites has been obtained from simple inhibition studies (Goldstein & Hayes, 1978; Pereira & Kabat, 1979). From the physicochemical point of view, however, the recognition sites of most of these proteins are still incompletely characterized, and more detailed information from different techniques is needed for an understanding of the mechanism of carbohydrate binding.

The dynamics of lectin–saccharide interactions have been the subject of only a few investigations. NMR has been used to study the binding kinetics of glucosides and mannosides to concanavalin A (Brewer et al., 1972; Grimaldi & Sykes, 1975) and to probe the nature of *N*-acetylglucosamine bound to wheat germ agglutinin (Neurohr et al., 1980a). In addition, stopped-flow measurements on the binding of 4-methylumbelliferyl and *p*-nitrophenyl derivatives of mannopyranosides and glucopyranosides to concanavalin A have been reported, most recently by Van Landschoot et al. (1980a) and Farina & Wilkins (1980), and also a T-jump relaxation study on the binding of *p*-nitrophenyl β-D-galactopyranoside to the lectin from *Ricinus communis* (RCA_I) (Podder et al., 1978).

We recently reported the determination of association constants and thermodynamic parameters for the binding of sugars to peanut agglutinin (PNA)[1] by UV difference spectroscopy (Neurohr et al., 1980b). This protein is a tetramer, of 27 500 protomer weight (Lotan et al., 1975); it has four binding sites and contains one atom each of Ca and Mg per subunit (Neurohr et al., 1980b). Peanut agglutinin is specific

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[1] Abbreviations used: PNA, peanut agglutinin; PBS, phosphate-buffered saline (0.01 M phosphate and 0.15 M NaCl, pH 7.2).

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for D-galactosyl end groups (Lotan et al., 1975) and recognizes the T antigen. It has been widely employed in studies of cell surfaces (Sharon, 1980). To obtain the kinetic data required for a complete picture of the mechanism of sugar binding by PNA, we have studied the interaction of the methyl α- and β-D-galactopyranosides with this lectin by carbon-13 NMR spectroscopy by use of methyl galactosides specifically labeled with ^{13}C at the anomeric carbon. Reaction rate constants and activation parameters (activation enthalpy, entropy, and free energy) are presented. The data indicate an entropy-controlled association process. They are interpreted in terms of possible binding mechanisms, consistent also with UV difference and CD spectroscopic data, and are compared with the corresponding parameters reported for the binding kinetics of sugars to concanavalin A.

Materials and Methods

PNA was purified by affinity chromatography on a lactose-based affinity matrix, as described previously (Neurohr et al., 1980b). The lectin was eluted from the column with 0.1 M D-galactose. Protein concentrations were determined by absorption, using $A_{280}^{1\%} = 7.7$ (Lotan et al., 1975). D-[1-^{13}C]Galactose (90% enriched) was a gift from Dr. H. C. Jarrell of these laboratories.

Synthesis of Methyl α- and Methyl β-D-[1-^{13}C]Galactopyranoside. These compounds were synthesized by reacting 90% D-[1-^{13}C]galactose with methanol according to the following procedure: 300 mg of D-[1-^{13}C]galactose was dissolved in 20 mL of methanol containing 280 μL (7%) of trifluoromethylsulfonic acid as catalyst, and the mixture was refluxed overnight. These conditions, chosen from preliminary experiments, lead to the formation of significant amounts of the β-pyranoside besides the main product, the α-pyranoside, and only small amounts of furanosides. The acid catalyst was removed by stirring the mixture with Rexyn 201 (OH^-) ion-exchange resin (Fisher Scientific Co., Ltd.) for 15 min. The resin was filtered off and washed with methanol. The combined methanol solutions were evaporated to dryness, and the residue was taken up in 3 mL of water. The isomeric methyl galactosides were separated by ion-exchange chromatography on Dowex-1 (OH^-) according to the procedure of Austin et al. (1963). The elution was monitored with a differential refractometer (Waters Scientific Ltd.). Fractions containing the α-pyranoside, and those containing the β-pyranoside, were pooled and lyophilized.

NMR Measurements. NMR samples were made up in PBS buffer containing 0.02% sodium azide at pH 7.2. The protein concentration was 0.05, 0.075, or 0.085 mM in PNA tetramer. The concentration of the 1-^{13}C-enriched glycosides varied between 0.4 and 10 mM. *p*-Dioxane was used as an internal standard. All samples contained 20% D_2O in order to provide a lock signal. ^{13}C NMR spectra were recorded in 10-mm tubes on a Bruker CXP-300 spectrometer at 75.5 MHz, using quadrature detection and broad-band proton decoupling. The temperature of the probe was maintained with a Bruker BVT 1000 temperature unit and monitored with a thermocouple in the probe. Line widths measured at half-height of the ^{13}C resonance of the enriched carbon of the sugars were corrected for magnetic field inhomogeneity, using the *p*-dioxane half-width as the correction factor.

Results

Figure 1 shows the ^{13}C resonance of 90% methyl β-D-[1-^{13}C]galactopyranoside in the presence and absence of peanut agglutinin. In the presence of PNA, the ^{13}C resonances of the enriched carbons of methyl α- and methyl β-D-galactopyranoside show line broadening, as displayed in Figure 1 for the β anomer. No chemical shift changes were observed for the ^{13}C resonances of both anomers upon binding to the protein. Due to the high enrichment with ^{13}C (90%) and the high field employed, very good signal to noise ratios could be obtained, even at the lowest sugar concentrations used in the present study. Since the observed line-broadening effects are relatively small, several control experiments were carried out in order to ascertain that they are due to specific binding of the sugars to the protein: (i) the line broadening, $\Delta\nu$, as displayed in Figure 2, is a linear function of the fraction bound, f, calculated from the equilibrium constants obtained by UV difference spectroscopy (Neurohr et al., 1980b); (ii) the total sugar concentration, [S], is a linear function of the reciprocal

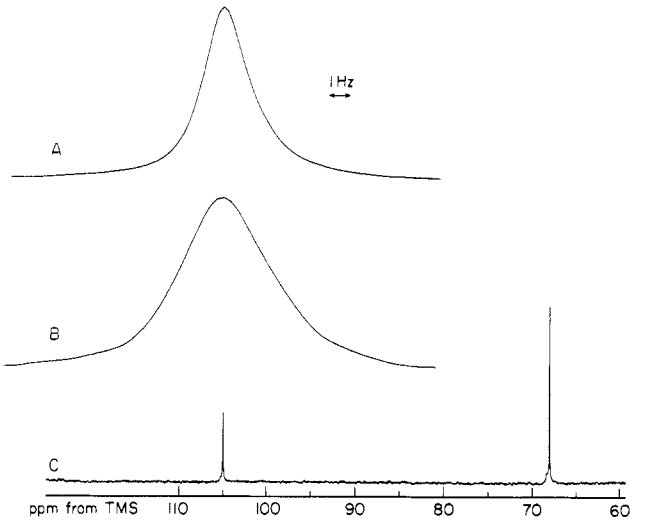

FIGURE 1: Proton-decoupled ^{13}C NMR spectra of methyl β-D-galactopyranoside (90% ^{13}C at C-1) in the absence and presence of PNA. (A) Spectrum of the free sugar in PBS at pH 7.2 and 25 °C; (B and C) corresponding spectra of 7.5×10^{-4} M methyl β-D-galactopyranoside (B) and 4×10^{-4} M methyl β-D-galactopyranoside (C) in the presence of 8.7×10^{-5} M PNA tetramer. Spectra A and B are shown on an expanded scale. Digital line broadening of 2 Hz was applied in each case. The resonance at high field in (C) is due to the *p*-dioxane internal standard.

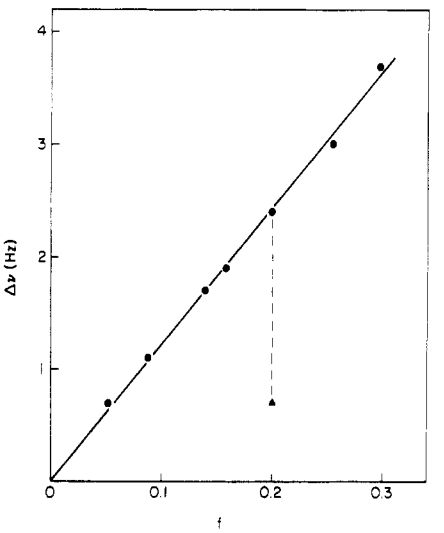

FIGURE 2: Plot of the change in C-1 line width ($\Delta\nu$) of 1-^{13}C-enriched methyl β-D-galactopyranoside in the presence of 8.7×10^{-5} M PNA as a function of f, the fraction of sugar bound at 25 °C. The total sugar concentration varied from 3.8×10^{-4} to 6×10^{-3} M. Spectra were obtained in PBS at pH 7.2 and 25 °C. (▲) The line-width change in a sample containing 7.5×10^{-4} M 1-^{13}C-enriched methyl β-D-galactopyranoside, to which was added an excess (10 mM) of unlabeled methyl α-D-galactopyranoside.

Table I: Temperature Dependence of the Lifetime in the Bound State (τ_M), Dissociation Rate Constant (k_{diss}), Equilibrium Constant (K_A), and Association Rate Constant (k_{assoc}) for the Binding of Methyl α- and β-D-Galactopyranoside to PNA[a]

	methyl α-D-galactoside				methyl β-D-galactoside			
t (°C)	$\tau_M \times 10^2$ (s)	k_{diss} (s^{-1})	$K_A \times 10^{-3}$ (M^{-1})	$k_{assoc} \times 10^{-4}$ (M^{-1} s^{-1})	$\tau_M \times 10^2$ (s)	k_{diss} (s^{-1})	$K_A \times 10^{-3}$ (M^{-1})	$k_{assoc} \times 10^{-4}$ (M^{-1} s^{-1})
5	11.5	8.7	5.6	4.9	10.8	9.2	3.6	3.3
10	8.7	11.5	4.0	4.6	6.9	14.5	2.5	3.6
15	6.2	16.2	2.7	4.5	4.7	21.3	1.8	3.8
20	5.0	20.1	2.2	4.5	3.5	28.7	1.3	3.6
25	3.6	27.6	1.8	4.9	2.5	39.4	0.94	3.7
30	3.0	33.4	1.3	4.3				
35	2.2	45.5	1.0	4.3				

[a] All data were obtained in PBS buffer, pH 7.2; estimated errors of τ_M, k_{diss}, and K_A are ca. ±15%, those of k_{assoc} ca. ±30%.

line broadening, $1/\Delta\nu$, when plotted according to eq 2 (see below); (iii) in the presence of an excess of another sugar, which binds to the protein, the line broadening is strongly reduced, as shown in Figure 2; (iv) upon thermal denaturation of the protein, the line-broadening effects disappear. These experiments clearly show that the observed line-broadening effects are due to specific binding of the glycosides to the protein. Moreover, the equilibrium constants obtained here from NMR titrations are in very good agreement with those obtained previously by UV difference spectroscopy (Neurohr et al., 1980b).

Broadening of the NMR of a small molecule due to partial binding to a protein can be treated by the method of Swift & Connick (1962). The observed spin–spin relaxation rate, $1/T_{2\,obsd}$, for a nucleus undergoing chemical exchange between the free and protein-bound states is given by eq 1

$$\frac{1}{T_{2\,obsd}} = \frac{1}{T_{2\,free}} + \frac{f}{T_{2M} + \tau_M} \qquad (1)$$

where $1/T_{2\,free}$ is the relaxation rate of the free sugar, T_{2M} is the spin–spin relaxation time in the bound environment, f is the fraction of sugar bound, and τ_M is the lifetime in the bound state ($1/k_{diss}$). This equation assumes that the chemical shift difference between the free and bound states is negligible and that the free sugar is in excess over the bound sugar ($f \leq 0.3$). In this case, only a single resonance is observed in the NMR spectrum, broadened due to exchange with a small fraction of bound sugar. In the fast exchange limit ($T_{2M} \gg \tau_M$), one observes the average width of the free and the bound sugar, while in the slow exchange limit ($\tau_M \gg T_{2M}$), the line broadening is governed by the exchange rate $1/\tau_M$, which is equal to the dissociation rate constant, k_{diss}, of the sugar–protein complex. It is possible to distinguish between these two cases by using the temperature dependence of the line broadening. For a variety of relaxation mechanisms, $1/T_{2M}$ is a monotonically increasing function of the correlation time of the sugar–protein complex, and the correlation time decreases upon temperature increase. T_{2M}, therefore, increases with temperature. The residence time of the sugar on the protein, τ_M, on the other hand, decreases upon temperature rise.

Between 5 and 35 °C, the 1-^{13}C resonance of the α anomer of methyl D-galactopyranoside in the presence of PNA broadened with increasing temperature, as shown in Figure 3. In this temperature region, therefore, the α anomer is in slow exchange on the ^{13}C NMR time scale between free and protein-bound environments, and the line-broadening effects are governed by the residence time of the sugar on the protein ($\tau_M \gg T_{2M}$). In the case of the β anomer of methyl D-galactopyranoside, slow exchange conditions were found to prevail up to 25 °C. In these temperature regions, therefore, the residence times τ_M and their inverse values, the dissociation

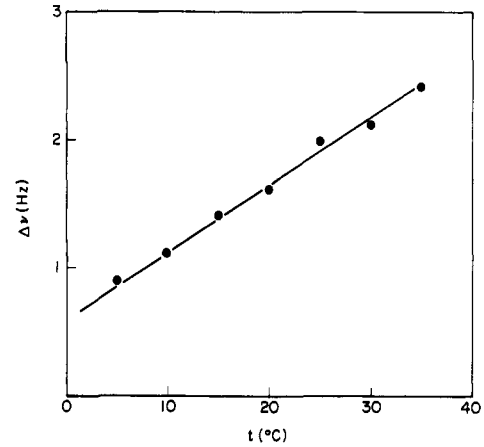

FIGURE 3: Variation of the line broadening of the C-1 resonance of 90% methyl α-D-[1-^{13}C]galactopyranoside in the presence of PNA as a function of temperature at pH 7.2. The concentrations used were 7.5×10^{-4} M methyl α-D-galactopyranoside and 7.5×10^{-5} M PNA tetramers.

rate constants, as well as the dissociation equilibrium constants of the protein–sugar complex, can be determined from a study of the line widths of the 1-^{13}C resonances of the two sugars in the presence of the protein. For an excess of sugar, $[S] \gg n[P]$, the concentration of the sugar–protein complex is given by

$$[SP] = \frac{n[P][S]}{K_D + [S]}$$

where $n[P]$ is the concentration of protein binding sites, [S] is the total sugar concentration, and K_D represents the dissociation constant of the sugar–protein complex. With $f = [SP]/[S]$ and $\Delta\nu = \Delta\nu_{obsd} - \Delta\nu_{free} = 1/\pi T_{2\,obsd} - 1/\pi T_{2\,free}$, where $\Delta\nu$ is the line width in hertz, one obtains eq 2. A plot

$$[S] = \frac{1}{\Delta\nu}\frac{n[P]}{\pi\tau_M} - K_D \qquad (2)$$

of [S] vs. $1/\Delta\nu$ yields the dissociation constant K_D from the y intercept, while the slope yields the residence time, $\tau_M = 1/k_{diss}$. The number of carbohydrate binding sites on the PNA tetramer, n, was found to be 4 (Neurohr et al., 1980b). Figure 4 shows a typical plot of [S] vs. $1/\Delta\nu$, according to eq 2, for the binding of the β anomer to PNA at 25 °C. Similar plots are obtained at other temperatures, and for the binding of the α anomer to PNA between 5 and 35 °C. Residence times, τ_M, obtained from the slopes of these plots and the corresponding dissociation rate constants, k_{diss}, for the binding of the two galactopyranosides to PNA at different temperatures are summarized in Table I. Experiments were carried out at different protein concentrations, 0.05, 0.075, and 0.085 mM in PNA tetramer. Within experimental error, the same values

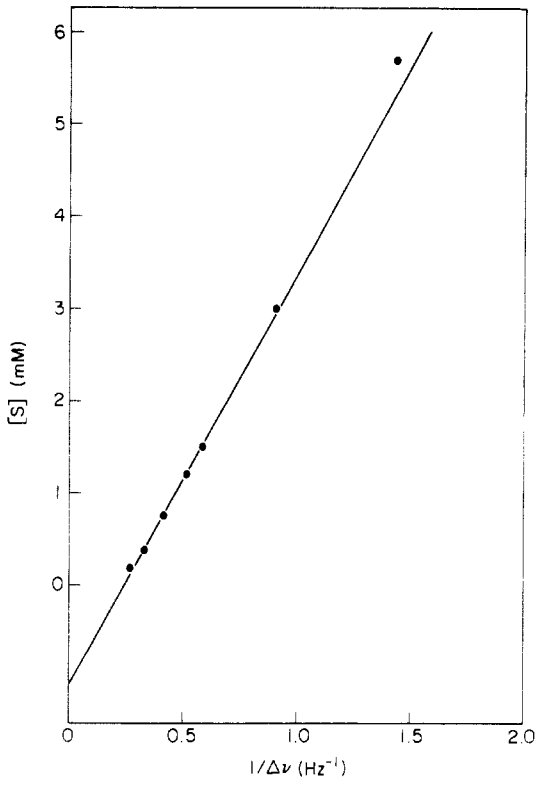

FIGURE 4: Plot of the total sugar concentration, [S], vs. the reciprocal line broadening, $1/\Delta\nu$, according to eq 2 for the binding of methyl β-D-galactopyranoside to PNA at pH 7.2 and 25 °C.

for τ_M and K_D were found, indicating that the observed parameters do not depend on the protein concentration used in the experiments. Equilibrium constants, K_A, as obtained from the y intercepts (which give $K_D = 1/K_A$), are included in Table I. Since the equilibrium constant is the ratio of association and dissociation rate constants ($K_A = k_{assoc}/k_{diss}$), the association rate constants at different temperatures can also be calculated and are shown in Table I. As mentioned earlier, the K_A values obtained here from NMR studies are in good agreement with those determined previously by UV difference spectroscopy (Neurohr et al., 1980b). The total enthalpy change, ΔH°_{total}, and the total entropy change, ΔS°_{total}, on binding, as obtained from the temperature dependence of these constants via a van't Hoff plot (and $\Delta G^\circ_{total} = \Delta H^\circ_{total} - T\Delta S^\circ_{total}$), are therefore also in good agreement with corresponding parameters determined by UV spectroscopy. From the temperature dependence of the association and dissociation rate constants, the activation energy E_A can be calculated from an Arrhenius plot, as shown in Figure 5. The activation parameters $\Delta H^\ddagger$ (enthalpy), $\Delta S^\ddagger$ (entropy), and $\Delta G^\ddagger$ (free energy) can then be calculated from the following equations (Laidler, 1979):

$$\Delta H^\ddagger = E_A - RT \quad (3)$$

$$k = \left(\frac{k'T}{h}\right) e^{\Delta S^\ddagger/R} e^{-\Delta H^\ddagger/RT} \quad (4)$$

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (5)$$

where k is the rate constant, k' is the Boltzmann constant, and h is Planck's constant. The values obtained for these parameters are listed in Table II.

Discussion

The control experiments carried out clearly show that the observed line-broadening effects are due to specific binding

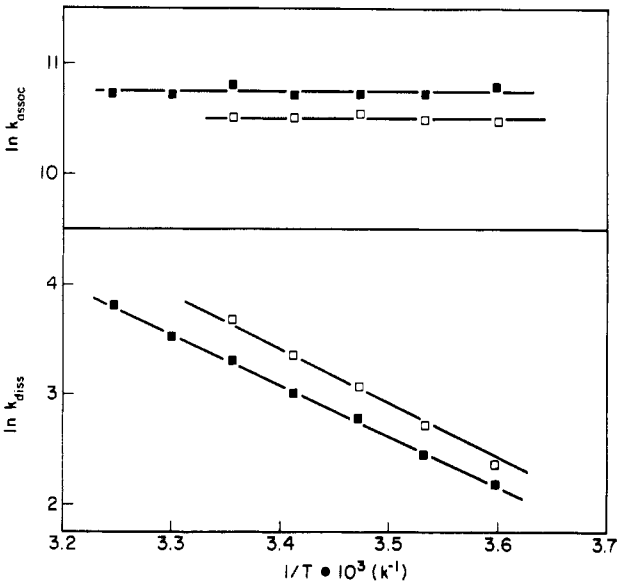

FIGURE 5: Arrhenius plots of the association and dissociation rate constants for the binding of methyl α-D-galactopyranoside (■) and methyl β-D-galactopyranoside (□) to PNA at pH 7.2.

Table II: Thermodynamic and Kinetic Data for the Binding of Methyl α- and β-D-Galactopyranoside to PNA[a]

parameter	methyl α-D-galactopyranoside (kcal mol⁻¹)	methyl β-D-galactopyranoside (kcal mol⁻¹)	source of data
ΔH°_{total}	−9.9	−10.2	T dependence of equilibrium constants (UV or NMR)
$E_{A\ assoc}$	~0	~0	Arrhenius plot of k_{assoc}
$E_{A\ diss}$	9.5	10.8	Arrhenius plot of k_{diss}
$\Delta H^\ddagger_{assoc}$	~0	~0	calcd from $E_{A\ assoc}$, eq 3
$\Delta H^\ddagger_{diss}$	8.9	10.2	calcd from $E_{A\ diss}$, eq 3
$T\Delta S^\circ_{total}$[b]	−5.6	−6.1	$\Delta G^\circ_{total} = \Delta H^\circ_{total} - T\Delta S^\circ_{total}$
$T\Delta S^\ddagger_{assoc}$[b]	−11.4	−11.0	calcd from k_{assoc}, eq 4
$T\Delta S^\ddagger_{diss}$[b]	−6.6	−5.1	calcd from k_{diss}, eq 4
ΔG°_{total}[b]	−4.4	−4.1	equilibrium constant $\Delta G^\circ_{total} = -RT \ln K_A$
$\Delta G^\ddagger_{assoc}$	11.4	11.0	$\Delta G^\ddagger_{assoc} = \Delta H^\ddagger_{assoc} - T\Delta S^\ddagger_{assoc}$
$\Delta G^\ddagger_{diss}$	15.5	15.3	$\Delta G^\ddagger_{diss} = \Delta H^\ddagger_{diss} - T\Delta S^\ddagger_{diss}$

[a] $\Delta G^\circ_{total} = \Delta G^\ddagger_{assoc} - \Delta G^\ddagger_{diss}$; $T\Delta S^\circ_{total} = T\Delta S^\ddagger_{assoc} - T\Delta S^\ddagger_{diss}$; $\Delta H^\circ_{total} = \Delta H^\ddagger_{assoc} - \Delta H^\ddagger_{diss}$. Estimated errors are ±1 kcal mol⁻¹ except for ΔH° and ΔG° (0.1 kcal mol⁻¹) and $\Delta G^\ddagger$ (2 kcal mol⁻¹). [b] At 25 °C.

of the glycosides to the protein. The temperature dependence of the line broadening moreover indicates that the two anomers are in the regime of slow exchange with the protein. Residence times τ_M and the dynamics of sugar–lectin interactions can therefore be obtained from the ^{13}C line widths of the sugars in the presence of the protein. Although rate constants generally can be obtained more accurately from chemical relaxation measurements, the bulky labels used in T-jump and stopped-flow experiments on lectin–saccharide kinetics so far (Clegg et al., 1977; Williams et al., 1978) may significantly alter the binding mechanism (see below), as comparison of K_A

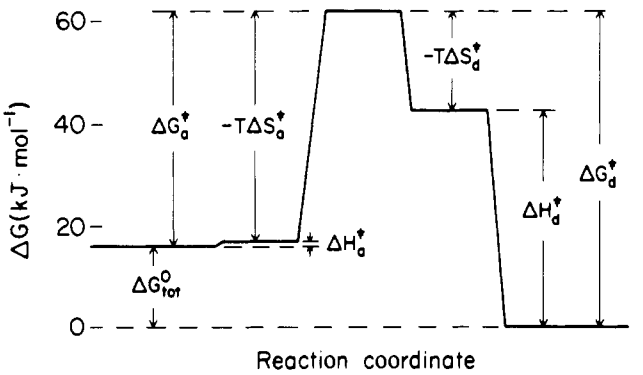

FIGURE 6: Thermodynamic/kinetic profile of the binding of methyl α-D-galactopyranoside to PNA at pH 7.2 and 25 °C.

values for the glycosides and parent oligosaccharides suggests (Van Landschoot et al., 1980b). The ^{13}C NMR study, on the other hand, has the advantage of using a nonperturbing label and is a valuable alternative method for kinetic investigations. The NMR parameters monitor directly the slower dissociation kinetics of the sugar–protein complex and yield dissociation rate constants and their activation parameters. Since the equilibrium constants are also obtained from the NMR measurements (and in the present case were in good agreement with those from UV measurements), the association rate constants ($k_{assoc} = K_A k_{diss}$) can also be calculated.

The activation parameters for the binding of the methyl galactopyranosides to PNA (Table II) show there is a considerable activation enthalpy for the dissociation reaction. The most striking feature of our results is that the association rate constants for both anomers are essentially temperature independent, implying that the association process occurs without a significant enthalpy barrier. The association rate constants are, however, much lower than those expected for a diffusion-controlled process. Rate constants for the formation of enzyme–substrate complexes are generally of the order of 10^6–10^7 M^{-1} s^{-1} (Hammes & Schimmel, 1970), which is also less than the theoretical diffusion limit. Inspection of the activation parameters in Table II shows that the association rate constant is limited by a free energy of activation, comprised essentially of an entropy term. The barrier for the association process between the lectin and the methyl galactopyranosides thus appears to be entropic, and not enthalpic.

Some checks of the overall validity of our data can be made. The total enthalpy change, $\Delta H°_{total}$, can be obtained from equilibrium measurements, e.g., from the temperature dependence of the equilibrium constants, as obtained from the NMR titrations (and also from previous UV measurements). The same parameter can also be calculated from the kinetic data, since $\Delta H°_{total} = \Delta H^{\ddagger}_{assoc} - \Delta H^{\ddagger}_{diss}$. As can be seen from Table II, both values determined independently are in good agreement for both sugars. Moreover, the total entropy change, $T\Delta S°_{total}$, as obtained from the UV equilibrium data (via $T\Delta S°_{total} = \Delta H°_{total} - \Delta G°_{total}$), is in good agreement with the value calculated from the NMR-derived activation entropies, $T\Delta S°_{total} = T\Delta S^{\ddagger}_{assoc} - T\Delta S^{\ddagger}_{diss}$ (Table II). Figure 6 shows a thermodynamic/kinetic profile of the binding of methyl α-D-galactopyranoside to PNA at 25 °C.

Our data indicate, therefore, that the association process between the lectin and methyl galactopyranosides is limited by a negative entropy of activation. This activation entropy for the binding reaction suggests that a particular configuration of reactants is required for sugar binding by the lectin. Like other plant lectins studied so far, PNA does exhibit a near-UV circular dichroic spectrum which undergoes saccharide-induced transitions in the presence of lactose (Fish et al., 1978). The near-UV CD changes observed with methyl α- and β-D-galactopyranoside are very similar to those observed with lactose (N. M. Young, K. J. Neurohr, and R. E. Williams, unpublished experiments). We also observed a change in the UV absorption of tyrosine residues upon binding of sugars to PNA. This enabled us to determine association constants and the thermodynamics of sugar binding to PNA by UV difference spectroscopy (Neurohr et al., 1980b).

The following binding model would be consistent with all of the various data: The first step, which is probably diffusion controlled (or nearly so), would be a loose association of the sugar with some residues in the binding site. This fast step would occur with a low equilibrium constant. The enthalpy change associated with this step would be small and difficult to detect, particularly if masked by a change of opposite sign arising from subsequent side-chain movements and/or hydration changes in the binding site. This step would not be detected by our NMR probe and would also not be observable in fluorescence or absorption T-jump or stopped-flow measurements. The second association step would be a mutual fitting of site and sugar. Our CD and UV spectroscopic results would then reflect protein conformational changes during this step. This conformational fitting we suggest would proceed through a highly ordered activated state and would therefore be associated with a negative entropy of activation. The process may involve movement of side chains in the active site and hydration changes, both being capable of giving the observed CD and UV effects. This mechanism is preferable to one in which the sugar is recognized by a minor protein conformer, since the plot of the equilibrium constants and the Arrhenius plots are linear with the reciprocal temperature over the temperature range studied, indicating no significant changes of the binding mechanism with temperature. A conformational equilibrium of the protein, on the other hand, should show a temperature dependence, if there is a significant enthalpy change involved. For the dissociation reaction, a considerable activation enthalpy is required to break the interactions between the sugar and protein side chains. In addition, a negative activation entropy is required in order to bring the reactants into the more highly ordered activated state described above for the second step of the association process (Figure 6). The equilibrium constants obtained from NMR and UV measurements would monitor the total association process. The very good agreement of these values from NMR and UV measurements indicates that both methods sense the same binding phenomenon.

The results obtained for PNA can be compared with those from concanavalin A. The binding kinetics of methyl α- and methyl β-D-glucopyranoside to concanavalin A have been studied by NMR (Brewer et al., 1972), and the rate constants reported by these authors, e.g., $k_{assoc} = 5 \times 10^4$ M^{-1} s^{-1} and $k_{diss} = 31$ s^{-1} for the α-glucoside; $k_{assoc} = 2.8 \times 10^4$ M^{-1} s^{-1} and $k_{diss} = 400$ s^{-1} for the β-glucoside (all at 25 °C in 0.1 M phosphate buffer at pH 5.6), are very similar to the values we find for the interaction of the methyl galactosides with PNA. Their data also indicate the absence of a significant activation energy for the association reaction. Quite different results, however, were obtained in stopped-flow absorbance and T-jump fluorescence studies, employing *p*-nitrophenyl α-D-mannopyranoside and 4-methylumbelliferyl α-D-mannopyranoside, respectively (Lewis et al., 1976; Clegg et al., 1977), and in a recent study with 4-methylumbelliferyl and *p*-nitrophenyl derivatives of α- and β-D-mannopyranosides and D-glucopyranosides (Farina & Wilkins, 1980). While the rate constants (association and dissociation) were in the same range

of values as obtained for the methyl glucosides by NMR (Brewer et al., 1972), a considerable activation enthalpy for the binding reaction was found, and the results were interpreted in terms of a simple bimolecular association step. A fast initial step like the one proposed here would not be observable with these techniques, since in the first encounter complex the labeled group would still be exposed to the solvent, and no spectroscopic change would occur. The considerable activation enthalpy for the association reaction in these studies may be due to the presence of the bulky reporter group, which may significantly change the binding mechanism, as the K_A values (Van Landschoot et al., 1980b) suggest.

It is noteworthy that the association rate constants found here for the binding of simple sugars by PNA, and previously for glycosides to concanavalin A [$(10^4-\sim 5 \times 10^5)$ M^{-1} s^{-1}], are below those reported for other carbohydrate-recognizing proteins, such as the murine myeloma proteins that bind galactose (Zidovetzki et al., 1980), and for enzymes in general (Hammes & Schimmel, 1970). This may be because these simple sugars are not the true ligands for which the lectins are specific in their natural role but are analogues of the true ligands. Complex glycopeptides often have greater K_A values for lectins than do the simple glycosides (Baenziger & Fiete, 1979); they have been found to give significantly different effects on concanavalin A, as observed by NMR (Brewer, 1979).

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