Anomer-Specific Recognition and Dynamics in a Fucose-Binding Lectin

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ABSTRACT: Sugar binding by a cell surface ~29 kDa lectin (RSL) from the bacterium Ralstonia solanacearum was characterized by NMR spectroscopy. The complexes formed with four monosaccharides and four fucosides were studied. Complete resonance assignments and backbone dynamics were determined for RSL in the sugar-free form and when bound to L-fucose or α-mannose. RSL was found to interact with both the α- and the β-anomer of L-fucose and the “fucose like” sugars α-arabinose and L-galactose. Peak splitting was observed for some resonances of the binding site residues. The assignment of the split signals to the α- or β-anomer was confirmed by comparison with the spectra of RSL bound to methyl-α-L-fucoside or methyl-β-L-fucoside. The backbone dynamics of RSL were sensitive to the presence of ligand, with the protein adopting a more compact structure upon binding to L-fucose. Taking advantage of tryptophan residues in the binding sites, we show that the indole resonance is an excellent reporter on ligand binding. Each sugar resulted in a distinct signature of chemical shift perturbations, suggesting that tryptophan signals are a sufficient probe of sugar binding.

Carbohydrate recognition is a topic of far-reaching importance1 that continues to challenge the protein scientist2−11 and the synthetic chemist12−16 alike. The principal challenge lies in understanding the balance of noncovalent interactions that favors receptor binding over solvation in water. Complex formation in the case of monosaccharides has the added feature of selection between the α and the β anomeric forms. NMR spectroscopy offers the powerful tools of chemical shift perturbation (∆δ) analysis to characterize protein−ligand binding. Here, we have applied this technique to study the fucose-binding lectin11 (RSL) from the bacterium Ralstonia solanacearum. Knowledge of fucose recognition by lectins is expected to aid the development of new strategies to combat bacterial disease. For example, host−pathogen interactions may be mediated by lectins that bind to fucosylated oligosaccharides present in plant cell walls or mammalian epithelial tissue.12 In the case of R. solanacearum, extracellular fucose-binding lectins likely contribute to infection and progression of wilt disease in potato and tomato.11

RSL is a ~29 kDa trimer with a six-bladed β-propeller fold.11 Each monomer comprises two blades, corresponding to the N- and C-terminal halves of the protein, which share ~40% sequence identity (Figure 1). There are two sugar-binding sites per monomer. Site 1 is intra-monomeric and is nestled between the N- and C-terminal blades of the monomer. Site 2 is inter-monomeric and occurs between the blades of adjacent monomers (Figure 1). Each site contains a WXGXGWX₅₀₅₀ motif. The indole ring of W₅ forms CH−π bonds13,14 with the monosaccharide, while the indole of W₄₅ donates a hydrogen bond to hydroxyl O₃ of fucose.15 The occurrence of two almost identical binding sites makes RSL an interesting model protein for ∆δ analysis. The protein is exceptionally stable (Tm = 86 °C in the sugar-free form),15 which favors NMR studies. There are numerous crystal structures (including PDB 2BT9 at 0.94 Å resolution) of RSL11 and related lectins15−18 in sugar-free and -bound forms. Furthermore, ligand binding to RSL has been characterized by isothermal titration calorimetry11,14 and computational methods.14,19,20

The interactions of RSL with four monosaccharides and four fucosides were investigated by chemical shift perturbation studies on ¹⁵N-labeled RSL. Remarkably, we observed anomer-specific effects with resonance splitting in the presence of L-fucose or related monosaccharides. While many lectin−carbohydrate complexes have been characterized by HSQC spectroscopy,16,21−30 we have not found evidence of such anomer-specific effects in the literature. NMR binding data for...
α- and β-methyl- and -nitrophenyl-β-fucosides together with analysis of the RSL crystal structure suggest that a Tyr side chain that flanks the intra-monomeric site may act as a steric impediment to the binding of β-1-fucoses. The backbone dynamics of the sugar-free and sugar-bound forms of RSL were characterized in the nanosecond to second time scales. Our data indicate an increased rigidity of the RSL structure upon sugar binding, consistent with a ligand-induced chemical shift perturbation. The assignments for RSL have been deposited in the BMRB with accession numbers 25950 (bound to D-mannose), 25951 (bound to L-fucose) and 25952 (sugar-free).

Sugar Binding and Chemical Shift Perturbations. Samples contained 0.25 mM 15N-RSL in 20 mM potassium phosphate, 50 mM NaCl pH 6.0 and 10% D2O. Monosaccharides or fucosides were added to a final concentration of 3 mM. Spectra were acquired on sugar-free RSL and sugar-bound RSL samples at 303 K on an Agilent 600 MHz NMR spectrometer equipped with a HCN cold probe. The differences in chemical shifts (\(\Delta\delta\)) between the sugar-free and the sugar-bound RSL were measured in CCPN, and the average perturbations were calculated as \(\Delta\delta_{\text{avg}} = \frac{1}{2} \left( \Delta\delta_{\text{f}} + (0.2 \times \Delta\delta_{\text{b}}) \right)\), \(\Delta\delta_{\text{f}}\) and \(\Delta\delta_{\text{b}}\) are the chemical shift differences between the sugar-free and sugar-bound spectra, respectively. The relaxation parameters and corresponding errors were extracted with CCPN, and the transverse relaxation rates were corrected for the \(R_{\text{ex}}\) contribution (see below). The diffusion tensors of the free and bound RSL were obtained from the experimental \(R_{\text{ex}}\) and \(R_{\text{r}}\) values using the program r2r1 diffusion (A. Palmer). Residues with a 15N \(R_{\text{r}}\) higher than one standard deviation from the average <\(R_{\text{r}}\)> (obtained for the entire set of the backbone amides) likely exhibit significant internal motions and were excluded from the analysis. The model selection was performed using F statistics as described elsewhere.

The CPMG relaxation dispersion experiments were recorded with 0, 25, 50 (in duplicate), 75, 125, 175, 225, 350, 550, 750 (in duplicate), and 1000 Hz pulse repetition rates. The peak intensities at each repetition rate were recorded with 0, 25, 50 (in duplicate), 75, 125, 175, 225, 350, 550, 750 ms. The ZZ exchange intensity build-up curves were analyzed using a composite ratio of four peak intensities (except Thr82) make at least one noncovalent bond with the sugar. Please refer to the DOI: 10.1021/acs.biochem.5b01212 Biochemistry 2016, 55, 1195–1203 for more details.

**EXPERIMENTAL SECTION**

**Materials.** Carbohydrates were purchased from SIGMA (D-arabinose, L-fucose, L-galactose, D-mannose), or Carbosynth (methyl-α-D-fucoside, methyl-β-D-fucoside, 4-nitrophenyl-α-D-fucoside and 4-nitrophenyl-β-D-fucoside). 13C-Labeled D-glucose and 15(NH4)2SO4 were purchased from Cortecnet.

**Protein Production.** 15N- and 13C/15N-RSL samples were produced in *Escherichia coli* BL21 transformed with the plasmid pET25rl1. Cultures were grown to mid-log phase on LB and then transferred to minimal medium (supplemented with 75 mg/mL carbenicillin). For 15N-labeling the medium contained 1 g/L 15(NH4)2SO4 as the sole nitrogen source. For 13C/15N-labeling 2 g/L 13C-labeled glucose was added as the sole carbon source. RSL was purified by affinity chromatography (D-mannose-agarose resin, SIGMA) on an AKTA FPLC, as described previously. Extensive dialysis was required to removed D-mannose. The protein concentration was determined by UV spectroscopy using an extinction coefficient ε280 = 44.6 mM M\(^{-1}\) cm\(^{-1}\).

**NMR Assignments.** The samples contained 2 mM 13C/15N-RSL in 20 mM potassium phosphate, 50 mM NaCl pH 6.1 and 6% D2O for the lock. The sugar-bound samples were prepared by the addition of D-mannose or L-fucose to a final concentration of 24 mM (i.e., 2 equiv). NMR experiments were performed at 303 K on Varian NMR Direct-Drive System 600 and 800 MHz spectrometers, the latter equipped with a salt-tolerant 13C-enhanced PFG-Z cold probe. All NMR data were processed in NMRPipe\(^{33}\) and analyzed in CCPN.\(^{34}\) The assignment of the backbone resonances were determined from a set of two-dimensional (2D) 1H-15N HSQC and 3D HNCA,CB, CBCA(CO)NH, and HNCO experiments.\(^{35}\) The tryptophan indoles were assigned from a combination of 2D (HB/CB(CGCD)(HB)\(^{36}\) and three-dimensional (3D) 15N- and 13C-NOESY-HSQC spectra, that provide C\(^2\)/H\(^\alpha\) correlations and strong intraresidue H\(^\alpha\)-H\(^\alpha\) NOEs, respectively. Finally, the side chain NH\(_{2}\) resonances of asparagine and glutamine residues were assigned from the CBCA(CO)NH spectrum by correlating with the corresponding C\(^\alpha\)/C\(^\beta\) (Asn) or C\(^\beta\) (Gln) chemical shifts. The assignments for RSL have been deposited in the BMRB with accession numbers 25950 (bound to D-mannose), 25951 (bound to L-fucose) and 25952 (sugar-free).

## Figure 1. (A) The primary structure of RSL comprises an N- and C-terminal half, which share ~40% sequence identity. Colons and dots indicate identical and similar residues, respectively, as revealed by (B) a structural alignment. Residues belonging to the intra- and intermonomeric binding sites are shown in gray and black, respectively. The panel on the right shows how the methyl substituent of the sugar is exposed to the solvent. Side chains shown as sticks (except Thr82) make at least one noncovalent bond with the sugar.\(^{11}\)
where $I(t)$ is the corresponding peak intensity at the mixing time $t$ and $k_{on}$ and $k_{off}$ are, respectively, the association and dissociation rate constants for the exchange process. A good fit ($\chi^2_{\text{red}} = 0.00069$) was obtained for the A85 data, with $\zeta = 1.235 \pm 0.022$ s$^{-2}$. For a system in a two-state $A \leftrightarrow B$ equilibrium, the equilibrium constant is given by $K = [A]/[B] = k_+/k_-$, where $k_+$ and $k_-$ are first-order association and dissociation rate constants, respectively, and the relative populations of the two species, $[A]/[B]$, can be estimated from the ratio of the corresponding peak intensities in the fully relaxed HSQC spectrum. With $K = [A]/[B] = k_+/k_-$, we obtain $k_+ = 1.05 \pm 0.16$ s$^{-1}$ and $k_- = 1.17 \pm 0.20$ s$^{-1}$, yielding the final value of the exchange rate constant $k_{ex} = k_+ + k_- = 2.22 \pm 0.25$ s$^{-1}$.

## RESULTS AND DISCUSSION

### Backbone Resonance Assignments

The $^1$H-$^{15}$N HSQC spectrum, with a total of 105 well-resolved cross-peaks, confirmed that RSL is a symmetric trimer in solution. Despite the relatively high molecular weight (29.2 kDa) the protein yielded an excellent HSQC comparable to that of a 10 kDa protein. Almost complete $^1$H, $^{15}$N, C′, C′, and C′ assignments were determined for sugar-free RSL. Figure S1A shows the assigned $^1$H-$^{15}$N HSQC spectrum. Pairs of homologous residues, due to the sequence and structural similarity of the N- and C-terminal subdomains, had resonances with closely similar chemical shifts and line widths (Figure S1A; S9/S52, I16/I61, R17/R62, W31/W76, D32/D77, W36/W81, and A40/A85, F41/Y86, see also Figure 2). Interestingly, no peaks were observed for the glycines that occur in the W$_{X}$G$X$GW$_{I+S}$ motifs (residues 31–36 and 76–81, Figure 1), suggestive of exchange broadening for these turn residues.

### Chemical Shift Perturbation Due to Sugar Binding

Monosaccharide/fucose binding to RSL was in slow-exchange on the NMR time scale. The presence of $\alpha$-arabinose, $\alpha$-fucose, methyl-$\alpha$-fucose, methyl-$\beta$-fucose, nitrophenyl-$\alpha$-fucose, nitrophenyl-$\beta$-fucose, $\alpha$-galactose or $\beta$-mannose (with $K_d$ values ranging from $<1$–100 $\mu$M$^{11,15}$), resulted in distinct perturbations of the backbone $^1$H, $^{15}$N resonances (Figure 3). Complete backbone assignments were determined for RSL bound to $\alpha$-fucose or $\beta$-mannose (see Figure S1B,C, respectively for the assigned HSQCs). In the case of the other sugars, only the $^1$H–$^{15}$N HSQC spectrum was assigned.

The sugars $\alpha$-arabinose, $\alpha$-fucose, and $\beta$-galactose have identical stereochemistry and differ only in the nature of the C5 substituent; $-\text{H}$, $-\text{CH}_2$, and $-\text{CH}_3\text{OH}$, respectively (Figure 3). In the crystal structure of RSL bound to methyl-$\alpha$-fucose$^{11}$ the C6 methyl is inserted into a shallow hydrophobic pocket formed by the side chains of Trp10, Ile59, Ile61, and Trp76 in the intra-monomeric site or Trp53, Pro14, Ile16, and Trp31 in the inter-monomeric site (Figure 1). In addition to interactions with these side chains the methyl substituent is also in van der Waals contact with two water molecules. The nature of this portion of the binding site, in particular, the water accessibility suggests a molecular basis for the broad specificity of RSL. In the case of $\alpha$-arabinose the absence of the C6 methyl is likely compensated by the insertion of a water molecule (Figure 4B). When $\beta$-galactose binds, its hydroxyl substituent can orient toward the solvent and there are no unfavorable clashes. The tolerance of this hydrophobic pocket to polar substituents is evidenced by the binding of $\beta$-mannose. By virtue of their similar stereochemistry, $\alpha$-fucose and $\beta$-mannose can bind to the same lectins.$^{11,16,48}$ Specifically, the protein-sugar contacts mediated by hydroxyls O2, O3 and O4 of $\alpha$-fucose can be established likewise by the O4, O3, and O2 groups of $\beta$-mannose (Figure 3). Consequently, when $\beta$-mannose binds to RSL its anomeric hydroxyl is inserted into the hydrophobic pocket. And considering the stereochemistry of C5 in $\alpha$-fucose it is apparent that RSL must bind $\beta$-mannose. One might also speculate that the $\sim 50$ fold lower affinity for $\beta$-mannose (compared to $\alpha$-fucose) is the result of a weakened hydrophobic effect at this site.

In the presence of $\alpha$-arabinose, $\alpha$-fucose or $\beta$-galactose, the number of resonances in the $^1$H–$^{15}$N HSQC spectrum of RSL increased by $\sim 10\%$. The assignment of RSL bound to $\alpha$-fucose revealed that the backbone amide resonances of six residues (Y37, F41, K83, G84, A85, and Y86) were split into two clearly resolved peaks. These pairs of resonances were attributed to the binding of the $\alpha$ and $\beta$ forms of $\alpha$-fucose, which is present in solution in a ratio of 0.3 $\alpha$-$\alpha$-fucose to 0.7 $\beta$-$\beta$-fucose. When bound to RSL, $\alpha$-fucose is positioned with the anomeric hydroxyl exposed to the solvent.$^{11,15}$ Thus, the $\alpha$ and $\beta$ forms can bind with minimal steric clashes between the protein and the anomeric hydroxyl. Crystallographic evidence for this effect was reported in a RSL crystal structure (PDB 4I6S), where both anomers of $\alpha$-fucose were bound.$^{15}$ As such RSL is suited to binding fucose-terminated oligosaccharides. While the lack of $\alpha/\beta$ anomer discrimination or selection is known for lectins,$^{16,17,47}$ we have not found NMR evidence for this effect in the literature. The assignment of the split peaks to the binding of the $\alpha$ and $\beta$ forms was confirmed by comparison with the spectra of RSL in complex with methyl-$\alpha$-$\alpha$-fucose or...
binding for this set of sugars were focused on the C5 stereoc- 
chery of four sugars. As discussed, the di-
verse interaction (e.g., hydrogen bonds and CH-
baric shifts), the use of uniFB00 samples (rather than uniform labeling) may provide a simpler probe for the characterization of sugar binding. An effective application

methyl-β-L-fucoside (Figure 2). There was good overlap between the fucose-split resonances and the single resonance in the fucoside complex. Note that the complex of RSL with d-
mannose did not result in peak splitting in the HSQC. Only the β-d-mannose can bind for reasons already discussed.

Pronounced chemical shift changes (Δδavg = 0.4–0.6 ppm) were measured for the resonances of equivalent residues Ala40 and Ala85 (Figure 3). These large effects can be rationalized by comparison with the X-ray structure.1 The amide NH of Ala40 and Ala85 donate a hydrogen bond to hydroxyl O2 of methyl-
α-L-fucoside (N→O2, 2.9 Å) in the intra- and inter-monomeric binding sites, respectively.

**Tryptophan Indole Probes of Sugar Binding.** The pattern of backbone chemical shift perturbations was similar for D-arabinose, L-fucose, L-galactose and D-mannose (Figure 3). Despite the differences in chemical structure, the main interactions (e.g., hydrogen bonds and CH→π bonds) were maintained by each sugar and the backbone resonances of RSL sensed similar changes to the chemical environment. In contrast, the –NH resonance of the tryptophan indole was a more sensitive reporter,1,32 and a unique pattern of shifts was observed for each monosaccharide. There are seven trypto-
phans in RSL, three in the intra-monomeric site (Trp10, Trp76, and Trp81), and three in the inter-monomeric (Trp53, Trp31, and Trp36). The seventh tryptophan (Trp74) does not contribute directly to either site.

Figure 4A shows the indole –NH perturbations in the presence of the four sugars. As discussed, the differences in binding for this set of sugars were focused on the C5 substituent (or C1 in the case of D-mannose, Figure 3). In the crystal structure, the methyl C6 of methyl-α-L-fucoside makes van der Waals contacts with the indoles of Trp 31/76 and Trp53/10. The shortest point of contact with C6 is to the indole –NH in the Trp31/76 pair (Figure 4B). Therefore, it could be expected that these indole resonances will have the largest differences in perturbation when the various sugars bind. This was clearly the case (Figure 4A, yellow bars). The Trp36/81 pair is furthest from C5, and the indole –NH of these side chains makes a hydrogen bond to hydroxyl O3, which is likely to dominate the chemical shift. Consistent with this structural interpretation, the Trp36/81 resonances had similar, large perturbations irrespective of which sugar was bound (Figure 4A, red bars). The Trp53/10 pair had similar perturbations for D-arabinose, L-fucose and L-galactose, while shifts of similar size but opposite sign were observed for D-mannose (Figure 4A, blue bars). Finally, the indole resonance of Trp74 had negligible chemical shift perturbations as this group was not involved in sugar binding. As with the backbone amide resonances, some of the indole resonances were also split due to the binding of α- or β-L-fucose (Figures 2C and 4A). Again the assignment of the split signals was confirmed by comparison with the data for methyl-α- or methyl-β-L-fucoside.

**Figure 4.** (A) Plot of chemical shift perturbations for the tryptophan indole resonance in the presence of four monosaccharides (refer to Figure 3). Filled and open circles correspond to the 1H and 15N data, respectively. Some resonances were split in two (e.g., L-fucose data) due to the presence of the α- and β-anomer. Blanks correspond to prolines (P14 and P44) and residues which lacked a resonance in the sugar-free or -bound RSL. The structure of each sugar and their Kd values1,15 are indicated. D-Mannose is oriented to show how hydroxyls O2, O3, and O4 match the stereochemistry of L-fucose.

Figure 3. Δδavg plots of RSL in the presence of 2 equiv of different monosaccharides. Black bars indicate resonances that were split in two due to the presence of the α- and β-anomer. Blanks correspond to prolines (P14 and P44) and residues which lacked a resonance in the sugar-free or -bound RSL. The structure of each sugar and their Kd values1,15 are indicated. D-Mannose is oriented to show how hydroxyls O2, O3, and O4 match the stereochemistry of L-fucose.
Steric Effects in the Binding Site. The observation of distinct chemical shift perturbations for the α- and β-anomers of L-fucose and methyl-L-fucoside prompted further analysis of this effect. In the atomic resolution crystal structure of RSL (PDB 2BT9) the methyl substituent of methyl-α-L-fucoside is solvent exposed (Figure 1B, right panel). The solvent exposure of the anomeric substituent implies that β-L-fucosides can be accommodated. However, the side chain of Tyr37 adjacent to the intra-monomeric binding site may impede the binding of bulky β-L-fucosides. The corresponding residue in the inter-monomeric site is Thr82 (Figure 1B). Figures 5 and S2 show overlaid spectral regions of RSL bound to nitrophenyl-α-L-fucoside, nitrophenyl-β-L-fucoside or the corresponding methyl-L-fucoside. In some cases the same chemical shift perturbation (with respect to sugar-free RSL) was observed for the α- and β-, methyl- or nitrophenyl-L-fucoside. For other resonances, the β-L-fucosides gave rise to perturbations that differed significantly from the α-L-fucoside shifts. For example, the R62 resonance was similarly altered in the presence of all four fucosides while the equivalent R17 in the intra-monomeric site was not shifted by the β-anomers. While it is difficult to pinpoint chemical shift changes to specific structural effects (due to the proximity of the intra- and inter-monomeric sites) it seems reasonable to assume that steric interactions between the β-substituent and the Tyr37 side chain (and the lack of such an interaction at Thr82) results in a different contribution to the chemical shift perturbations of binding site resonances. A role for tyrosine side chains in lectin selectivity has been proposed previously, for example, the tyrosine gate in fimH.5,6 Perhaps, the single Tyr37 serves to modulate the binding affinity of the three intra-monomeric sites in RSL.

Dynamics of Sugar-Free and Sugar-Bound RSL. The dynamics of the RSL backbone on the ps-ns time scale were assessed by using the residue-based squared-order parameter ($S^2$). With an average $S^2 = 0.82 \pm 0.08$, the sugar-free RSL backbone was rigid, except for the homologous loop regions comprising residues 32–35 and 77–80 (Figure 6, upper panels and compare Figure 1). These considerably flexible ($S^2 < 0.75$) loops contain two glycines (no amide resonances detected) and connect two of the sugar-binding tryptophans in the intra- and inter-monomeric binding sites. The flexibility of these loops was not affected by binding to either L-fucose or D-mannose. Residues 40–44 and 64–72 exhibited minor changes in the order parameters ($\Delta S^2 < 0.05$), while $S^2$ for the rest of the backbone remained constant. These data suggest that sugar binding did not affect the RSL backbone dynamics on the ps-ns time scale.

To complement the $S^2$ analysis, $^{15}$N $R_1$ and $R_2$ relaxation parameters were also measured. Governed by the rotational diffusion of a molecule, the $R_1$ and $R_2$ relaxation rates can provide information on the hydrodynamic properties of the protein. A small, uniform decrease in the $R_2/R_1$ ratios was observed for the sugar-bound forms of RSL compared to sugar-free RSL (Figure 6, middle panels). Quantitative analysis of the $R_2/R_1$ profiles yielded isotropic diffusion tensors with rotational correlation time $\tau_c = 12.42$ or 11.75 ns for the sugar-free and -bound RSL, respectively.9 These data are consistent with an RSL trimer in solution and suggest that the protein adopts a more compact structure upon sugar binding.

RSL dynamics were probed further by Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion spectroscopy.50,51 In this experiment, protein groups that undergo conformational exchange on the µs-ms time scale give rise to relaxation dispersion curves, which provide an exchange contribution ($R_{2ex}$) to the transverse relaxation rate $R_2$. In sugar-free RSL, ~40% of residues experienced $R_{2ex} > 0$ (Figure 6, lower panels), suggesting that the protein was in conformational exchange between two or more lowly populated species.
The clustering of these exchange effects suggests the possibility of concerted motions. Mannose binding moderately altered the distribution of the $R_{2ex}$ terms, with a reduction in the number of residues with $R_{2ex}$ contributions. In contrast, fucose binding abolished the $R_{2ex}$ terms for most of the protein. Such a drastic decrease in $R_{2ex}$ values suggests that the conformational dynamics observed in sugar-free RSL were quenched upon fucose binding. These data, together with the $\tau_c$ results, indicate an increased rigidity of the sugar-bound protein.

Finally, by using $^{15}$N ZZ-exchange spectroscopy, we studied the slow dynamics of RSL bound to the $\alpha$- and $\beta$-anomer of L-fucose. In this experiment, magnetization arising from species in slow exchange ($1 s^{-1} \leq k_{ex} \leq 10^3 s^{-1}$) gives rise to symmetry-related cross-peaks. The buildup of the cross-peak intensities, followed in a series of spectra recorded with varying mixing time, can be used to extract the kinetic parameters. At long mixing times (0.5–1 s), the resonances of RSL bound to $\alpha$- or $\beta$-L-fucose featured distinct cross-peaks (Figure 7), indicating that the two bound forms were in exchange. Only the A85 resonances were resolved sufficiently for quantitative analysis (Figure 7C) and an exchange constant $k_{ex} = 2.22 \pm 0.25 s^{-1}$ was obtained (see Experimental Section). Consistent with the $\mu$M binding affinity, the NMR data suggest that the $\alpha$- and $\beta$-L-fucose-bound forms of RSL undergo very slow exchange.

The dynamics of sugar-binding proteins have been assessed in numerous studies, some of which have pointed to a role for conformational entropy. In some examples, it was found that the sugar-bound form was more dynamic than the sugar-free form, with the entropy gain being thermodynamically favorable for ligand binding. In the case of RSL, the protein dynamics were reduced in the sugar-bound form. Upon binding to L-fucose the protein became more compact (decreased $\tau_c$) and motions on the ms-ms time scale were frozen out. Interestingly, the fast dynamics analysis revealed flexible loops ($S^2 < 0.75$) adjacent to both the intra- and inter-monomeric binding sites. Here, the flexibility was not affected by binding to monosaccharides. These loops extend away from the binding site and may play a structural role in RSL interactions with cell surface oligosaccharides.

**CONCLUSIONS**

Using NMR spectroscopy, we have shown that the bacterial lectin RSL binds both the $\alpha$- and the $\beta$-anomer of L-fucose and “fucose like” sugars. Anomer-specific chemical shift perturbations were observed for methyl- and nitrophenyl-substituted L-fucosides. A $^{15}$N ZZ-exchange experiment confirmed that RSL binds to $\alpha$- or $\beta$-L-fucose, which exchange on the second time scale. This appears to be the first example of such anomer-specific interactions measured by NMR. Analysis of the atomic
resonance is an excellent reporter of ligand binding was accompanied by decreased backbone motions on ligand-induced more compact structure when bound to bulky substituents. The relaxation data reveal that RSL adopts a compact structure in the presence of sugar.

![Figure 7. Slow dynamics of RSL bound to l-fucose. Selected spectral regions of the 1H N ZZ-exchange experiments acquired with t_\text{ex}=0 \text{ s (black), 0.23 s (blue), and 0.5 s (red) showing the resonances of (A) W81 and W36 (indoles) and (B) A8S. Dashed rectangles connect the four exchange cross-peaks. Note the intensity decrease of the autpeaks (AA, BB) and the concomitant increase of the cross-peaks (AB, BA) at longer mixing times. (C) Quantitative analysis of the build-up curve for the exchange cross-peaks of A8S. See Experimental Section for the fit parameters.](Image)

resolution crystal structure suggests further that the accessibility of the intra- and inter-monomeric binding sites is different. A Tyr side chain (Tyr37) adjacent to the intra-monomeric may act as a steric impediment to the binding of \( \beta \)-L-fucosides with bulky substituents. The relaxation data reveal that RSL adopts a more compact structure when bound to \( L \)-fucose, suggesting a ligand-induced fit. The protein compaction due to sugar binding was accompanied by decreased backbone motions on the \( \mu \text{s}-\text{ms} \) time scale. Finally, in addition to the conventional analysis of backbone amide chemical shifts, we show that the tryptophan indole resonance is an excellent reporter of ligand binding, with characteristic \( \Delta \delta \) signatures observed for each sugar.

### ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b01212.

Iterative design of a helically folded aromatic oligoamide sequence for the selective encapsulation of fructose. J. Biol. Chem. 287, 4335–4347.


