Relative Effects of Ionic and Neutral Substituents on the Binding of an Oligosaccharide by a Protein

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This communication presents a systematic evaluation of changes in the $\Delta G°$ of oligosaccharide binding by a lectin that result from alterations in the chemical nature of a substituent that, in the complex, comes near but not necessarily in contact with the protein. These effects, which appear to arise from changes in hydration, are found to be of sufficient magnitude to be of major importance to the appreciation of the forces leading to molecular recognition in aqueous solution.

A lectin of *Griffonia simplicifolia* (GS-IV)[1] binds the Lewis b human blood group related tetrasaccharide α-L-Fuc-(1→2)-β-D-Gal-(1→3)-[α-L-Fuc-(1→4)]-β-D-GlcNAcOMe (Le^b-OMe, **1**) with $K_{assoc} = 4.4 \times 10^4$ ($\Delta G° = -6.3$ and $\Delta H° = -13.3$ kcal/mol).[2] Both the X-ray crystal structure of native GS-IV and its complex with **1** are solved to 2.0 Å resolution.[3] As is schematically illustrated in Figure 1, with $R^1 = R^2 = R^3 = R^4 = H$, five of the 10 hydroxyl groups of **1** remain in contact with the aqueous phase after the complex with GS-IV has formed.[3,4] The internuclear distances between the oxygen atoms of the hydroxyl groups at positions 6b, 3c, 3d, and 4d (Figure 1) and the nearest detectable protein atom are 3.62, 3.52, 3.01, and 2.88 Å from the amino acid residues Tyr 223 (C), Arg 48 (N), Trp 138 (N), and His 114 (N), respectively.[3b] Although these distances are small, both deoxygenation and *O*-methylation at each of these five positions had only modest effects on the extents of the binding reactions.[4] In the case of the 6a position, which in the complex is farthest from the protein [6.21 Å from Ser 49 (O)], neither deoxygenation nor *O*-mesylation had a significant effect on the thermodynamic parameters.[2] Therefore, the effects of substitutional changes at this position were not investigated. However, the other four hydroxyl groups all come to reside near the periphery of the lectin's combining site, and, as seen in Table 1, the introduction of a $-CH_2CH_2R$ group at one of these positions, depending on the chemical nature of R, produced differential changes in free energy of binding in the range –0.8 to 2.1 kcal/mol. Once the complex has formed, the hydrophobic or amphiphilic substituents listed in Table 1 are expected to extend their terminal groups, by way of the ethylenic bridge, from 4 to 6 Å either along or away from the surface of the protein. Although some of the inhibition data presented in Table 1 are clearly in accord with general expectation, detailed commentary is reserved for a full analysis once the thermodynamic parameters have been determined and, hopefully, X-ray crystal structures have been achieved. It is noteworthy that *O*-methylation of either position 6b or 4d moderatly strengthened the binding, and, indeed, the 6b,2c,3d,4d-tetra-*O*-methyl derivative of Le^b-OMe (**1** with $R^1 = R^2 = R^3 = R^4 = Me$) is bound 0.5 kcal/mol more strongly than **1**.[4] It may also be of interest to note that a comparison of the effects on binding of deoxygenation and *O*-methylation at the various hydroxyl positions of an oligosaccharide allow the chemical mapping of the region that is recognized and bound by the protein.[4,5]

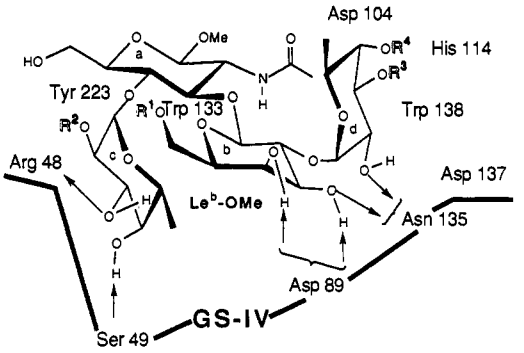

Figure 1. Schematic presentation of the complex of the Lewis b human blood group determinant, as its β-methyl glycoside [Le^b-OMe (**1**)] with the lectin IV of *Griffonia simplicifolia* (GS-IV). As indicated, the four R groups, which were modified to provide the data presented in Table 1, remain in contact with the aqueous phase. The other five hydroxyl groups become engaged, as illustrated, in hydrogen bonds with amino acid residues within and at the periphery of the combining site. The X-ray crystal structure of the complex has been published[4] with the *O*-methyl groups introduced at the five hydroxyl positions that remain in the aqueous phase in the orientations that appeared sterically most favorable on the basis of hard-sphere calculations.

Table 1. Effects of Mono-*O*-Substitution on the Extents of the Binding of the Tetrasaccharide Le^b-OMe by the Lectin GS-IV

	relative potency,[a] ($\Delta\Delta G°$, kcal/mol)[b] position substituted			
substituent	**6b** (R^1)	**2c** (R^2)	**3d** (R^3)	**4d** (R^4)
	Neutral			
$-CH_2CH_2Me$	3.30 (–0.7)	0.95 (0.0)	0.40 (0.5)	0.42 (0.5)
$-CH_2CH_2NHAc$	1.70 (–0.3)	0.54 (0.4)	0.54 (0.4)	1.21 (–0.1)
$-CH_2CHN(Me)Ac$	4.57 (–0.8)	1.00 (0.0)	0.13 (1.2)	1.29 (–0.1)
	Cationic			
$-CH_2CH_2NH_3^+$	0.88 (0.1)	0.04 (1.9)[c]	0.06 (1.7)[c]	0.17 (1.1)
$-CH_2CH_2NH_2Me^+$	1.11 (–0.1)	0.04 (1.9)	0.09 (1.4)[c]	0.18 (1.0)
$-CH_2CH_2NMe_3^+$	1.02 (0.1)	0.03 (2.1)[d]	0.05 (1.8)[c]	0.07 (1.6)[c]
	Anionic			
$-CH_2COO^-$	0.08 (1.4)	0.26 (0.8)	0.17 (1.1)[c]	1.39 (–0.2)[c]
$-CH_2CH_2COO^-$		0.60 (0.3)		

[a] Relative to the potency of Le^b-OMe set at 1.00 using an enzyme-linked immunosorbent assay (ELISA).[7] [b] $T \sim 23$ °C. [c] ±0.2. [d] ±0.4.

The differential changes in free energy caused by the substitutional changes were calculated from 50% inhibition data obtained by way of an enzyme-linked immunosorbent assay[6,7] (ELISA) based on the competition for GS-IV by the inhibitor with an artificial antigen [(Le^b-O$(CH_2)_8$CO)$_{14}$BSA].[8] The various inhibitors were synthesized from mono-*O*-allyl-per-*O*-benzyl derivatives of **1** which had been obtained according to procedures closely analogous to those used in the course of the preparation of mono-*O*-methyl derivatives of **1**.[4] The ^{13}C-NMR spectra at 75 MHz reflected high levels of purity and were in full accord with the structural assignments. The ^{1}H-NMR spectra at 360 MHz revealed in each case the specific interunit chemical shifts[9] that are characteristic of the conformaion of Le^b-OMe found in its complex with GS-IV.[4]

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As seen in Table 1, the introduction of an electrically neutral group can produce either an assistance or a hindrance to binding. Except for one of the substitutions, that at position 3d, all produced effects, apparently hydrophobic in character, of less than ±0.8 kcal/mol. With only one exception, substantial reduction in binding was observed as compared to the corresponding *n*-propyl compound when the substituent was ionic. The consistency of these ionic effects is particularly remarkable in view, as displayed in Figure 1, of the wide range of amino acid residues about the periphery of the combining site. The reductions in potency caused by ionic substituents, regardless of their environment in the complex, likely arise from the differences in the mobilities of the hydration shells about the ligand or over the surface of its complex with GS-IV.[11] The one exception involves the introduction of a carboxymethyl group at position 4d, which provides a complex that is 0.7 kcal/mol more stable than the corresponding *n*-propyl derivative. This result is likely attributable to the very close proximity of the imidazole group of His 114. On the other hand, the introduction of a carboxymethyl group at the 2c position (R^2 in Figure 1) caused a destabilization of 0.8 kcal/mol, although the anionic group comes to reside in close proximity to the cationic Arg 48 amino acid residue. The binding was strengthened by 0.5 kcal on lengthening the substituent to a carboxyethyl group.

Evidently, hydration effects can strongly influence the wide range of biological association that involve the accommodation of polar groups *not only within but also about the peripheries* of epitopes and their receptor sites. It is noteworthy in this regard that, as long anticipated, hydration effects have recently been experimentally justified in a number of different ways and, in fact, contribute importantly to the energetics of associations in aqueous media.[9,12-17] These observations are of obvious concern to the preparation of inhibitors of enzymes and adhesion molecules in general. Of course, similar considerations apply to the disposition of polar groups in the course of protein structures achieving the most favorable tertiary structures in the presence of water.

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Supplementary Material Available: Schemes showing the reaction pathways for **12**, **23**, **24**, and **25**, tables of ^{13}C chemical shifts and differential shifts and the effects of a mono-*O*-substitution on the extents of the binding of the tetrasaccharide Le^b-OMe by the lectin GS-IV, and a plot of the relative RIA vs ELISA potency (11 pages). This material is contained in many 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.

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